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ION EXCHANGE RESINS IN MEDICINE AND BIOLOGICAL RESEARCH

BY

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ION EXCHANGE RESINS IN MEDICINE AND BIOLOGICAL RESEARCH*

Conference Chairmen: H. SOBOTKA AND H. P. GREGOR
Consulting Editor: HARRY SOBOTKA

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* This series of papers is the result of a Conference on *Ion Exchange Resins in Medicine and Biological Research* held by the Section of Biology and Section of Physics and Chemistry of The New York Academy of Sciences, November 14 and 15, 1952.

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INTRODUCTION: ION EXCHANGE RESINS AND THEIR APPLICATION TO BIOLOGY AND MEDICINE

By Harry Sobotka and Harry P. Gregor

The Mount Sinai Hospital, New York, N. Y., and The Polytechnic Institute of Brooklyn, N. Y.

When we planned the conference on Ion Exchange Resins in Medicine and Biological Research, on which this monograph is based, it soon became apparent that the biochemical and medical applications of the ion exchange substances could best be presented within a wider framework including the preparative and physical chemistry of these compounds. Thus, Doctor Gregor undertook to organize the first half of the program, while Doctor Sobotka arranged its second moiety. We wish to thank, at this juncture, those who contributed their services as Session Chairmen: Doctors H. F. Mark, Polytechnic Institute of Brooklyn; Th. Shedlovsky, Rockefeller Institute for Medical Research; and Wm. Dock, State University of New York, who was the first physician to use ion exchange resins in patients.

In discussing the synthesis of ion exchange resins, Craig points out that the matrix of the resin is synthesized by either condensation polymerization or addition polymerization, and that substitution of the acidic or basic groups, which are responsible for the cation or anion exchanging properties, is usually effected by a separate procedure. In addition to the phenol-formaldehyde and styrene-divinylbenzene matrices, resins prepared from cellulose (in the form of cloth or yarn) and proteins are described. Doctor Craig sets forth methods for preparing resins of high as well as low density, the latter being the so-called "popcorn" polymers. Synthetic procedures for preparing resins having a variety of different exchange groups are outlined, and the general problem of preparing specific exchange resins is discussed. It is these specific resins which are potentially of greatest importance to biologists.

The present status of the problem of the synthesis and characterization of electron exchange polymers is presented by Ezrin and Cassidy. These new resins are prepared from vinylhydroquinone, and are the polymeric analogues of the quinone–hydroquinone redox system. Potentiometric titrations of the polymer, its use in chromatography when dispersed on filter paper, and its column behavior are described. This new material, which will effect redox reactions without being soluble itself, will find many important biological uses.

The general thermodynamic properties of ion exchange resin systems (in particular, their swelling properties as related to their ion selectivities) are discussed by Gregor and Frederick. The Gibbs–Donnan theory which applies to these systems is described. Free energies of swelling as evaluated from water sorption data are discussed and the calculation of approximate thermodynamic osmotic pressures is given. Where interactions exist between the resin or its fixed exchange group on the one hand and the exchanging ions on the other hand, it is shown that free energies of swelling are not reliable indications of ionic selectivity, but that volume changes *per se* are reliable indices usually.

The use of Harned's Rule in evaluating activity coefficients of ions in ion exchange resin systems is described by Davidson and Argersinger. This method is applied to exchange equilibria between hydrogen, sodium and silver ions in a sulfonic acid cation exchange resin system.

Soldano treats the kinetics of ion exchange processes, and discusses the effect of several factors upon rate processes. These include: (a) crosslinking, which slows the diffusion processes; (b) the influence of ionic charge; (c) the exchange capacity; and (d) the effect of resin composition. The diffusion of the solvent systems is described.

The general characteristics of cation exchange processes are described by Bregman, starting from a consideration of the resin systems as being cross-linked polyelectrolytes. The effect of several variables upon the selectivity of the resin is taken up, including (1) the resin matrix, (2) the acidic exchange group, (3) the fraction of the exchange capacity occupied by each cation, (4) the exchange capacity of the resin, (5) the ionic strength, and (6) the temperature of the ambient solution. The properties of a new phosphonic acid resin are described in terms of these considerations.

Anion exchange processes are described by Peterson, who first takes up the many applications of these resins, and then presents tables of data on anion selectivity coefficients. The sorption and exchange of several organic acids, many of these of biological importance, by various resins, are described.

A new process, termed ion exclusion, which involves the separation of ionic and non-ionic substances, is described by Wheaton and Bauman. Here one makes use of the different sorption affinities of various neutral molecules, *e.g.* glucose and methanol, for ion exchange resins. Separations are effected in most cases by merely eluting a sorbed mixture from a column with water. Many biologists will find this process of great interest.

The entire field of ion selective or ion exchange membranes is reviewed by Sollner, who has pioneered in this new field. The basic physical chemistry is discussed, and considerable experimental data on new and vastly improved membranes are presented. The exciting new applications to which these membranes apply is described by Doctor Sollner as well as their importance as models of living systems.

The application of ion exchange resins to biological and medical problems are manifold and may be classified as follows:

1. Chemical separations including uses in immunochemical problems;
2. Purification of pharmaceutical and biological preparations;
3. Therapeutic uses, primarily, the removal of sodium from the nutriment in conditions where a diet poor in sodium would be indicated;
4. Diagnostic applications.

In the field of the first two uses, Waldo Cohn gives a survey of biochemical separations on ion exchange columns. He reviews the work of Stone and Moore on amino acids and reports his own work and that of his collaborators on the separations of nucleotides and their smaller fragments. In the course of these experiments on the fractionation of chemical as well as enzymatic hydrolysates of nucleic acids, di-, tri-, and tetranucleotides were separated in addition to the known mononucleotides. The latter could be separated into three types of isomers to which specific structures have been assigned. This work is rounding out a concept of nucleic acid structure which is in excellent agreement with the theories developed with different techniques by W. R. Todd and others. Throughout these experiments and also in the work on sugars and sugar phosphates, described later in his report, Doctor Cohn emphasizes that the sequence of adsorption of the individual compounds is not merely governed by their pK-values, but also by other less predictable features such as aromatic side-chains and other steric factors. Thus, we find a certain overlapping of ion exchange with the older adsorption chromatography.

This trend is also apparent in the next contribution by Isliker who deals with the purification of antibodies by means of antigens linked to ion exchange resins. Here the ion exchange resins function as a matrix to which the antigen of an immunochemical system is irreversibly linked by certain chemical operations. The fixed stroma of blood corpuscles, for instance, acting as an insoluble antigen, becomes adsorbed to an ion exchange resin. It then adsorbs, in its turn, the isoagglutinin (antibody) from serum with an exceedingly high degree of specificity. The conditions for the dissociation between antigen and antibody, the competition between various agents and their vicarious replacements, throw much new light on the nature of the link between antigen and antibody. Practical usefulness of this knowledge in the service of the patient when he is to be given a blood or serum transfusion is evident.

Doctor Segal leads us through the diagnostic field and exemplifies the utilization of ion exchange resins pretreated with quinine, methylene blue, serenium, and fluorescein in an ingenious method which demonstrates and gauges the degree of acidity in the gastrointestinal canal without intubation by a simple test on the patient's urine. The use of the quininium indicator exchange resin in particular has reached the stage of practical routine application.

The rest of the monograph is devoted to the consideration of the important therapeutic uses of ion exchange resins. Some of these compounds are administered in sizable quantities to man, in order to reduce the amount of sodium available in the gastrointestinal tract for absorption from the nutriment. As it is the sodium ion which one wishes to remove, the work reported deals primarily with the effects of cation exchange resins. But Kohlstaedt and his collaborators and Danowski also evaluate the use of mixtures of anion and cation exchange resins. Heming and his colleagues report on the relative effectiveness of carboxylic, sulfonic and phosphonic type of resins and alginic acid and conclude that much work remains to be done before an ion exchange resin will become available that answers all requirements for satisfactory medication. Doctor McChesney and his coworkers favor the sulfonic type of ion exchange resins on theoretical as well as practical grounds.

The effectiveness of ion exchange therapy depends obviously on the amounts and concentration of ions to be exchanged. Their effect will be less tangible when the patient is on a strictly sodium-free diet to begin with; but we must recognize that the gastrointestinal tract is neither a glass pipe nor a cellophane tube, but is subject to numerous physiological influences and their pathological modifications. There is the concomitant regulation of potassium metabolism which can be neither ignored nor avoided, since the selectivity of ion exchange resins for sodium over potassium is limited. Doctors Danowski and Emerson, in their contributions, deal with the effect of the hormones of the adrenal cortex upon the transition of sodium and potassium through the intestinal wall from the blood into the lumen and *vice versa.* Doctor Levitt evaluates the effectiveness and indication of ion exchange resin therapy in its various cycles *i.e.* after pretreatment with the various ions. Doctor Visscher, a pioneer in this field, approaches the problem from the physiologist's angle with the most exact technique available. Using radioactive sodium from both sides, from the blood stream after injection or from the intestine after ingestion, he compares the rate of transition. His remarks throw much light on the reaction rate at various levels of the intestine and on the individual share of blood vessels and lymph vessels in the absorption process. The effect of ion exchange resins on organic cations, such as amines of physiological importance and on pharmaceuticals (*e.g.* digitalis) has not yet been considered. Dr. Levitt summarizes clinical experience in cardiac disease, acute renal insufficiency, and during cortisone and adrenocorticotrophic hormone therapy. Both he and Doctor Berger in his discussion remarks advise caution in the use of this powerful, but dangerous tool. Its use must be punctiliously followed by chemical analysis for the blood electrolytes and controlled by clear and intelligent reasoning on the part of the clinician.

SYNTHESIS OF ION EXCHANGE RESINS

By Paul N. Craig
Smith, Kline and French Laboratories, Philadelphia, Pa.

Although ion exchange agents such as the zeolites have been used as water softeners for many years, the advent of "tailor-made" synthetic ion exchange resins has opened entirely new fields for their use. The physical and chemical properties of such resins can be modified to fit particular requirements by the proper choice of reagents and conditions used either in the polymerization reactions or in subsequent chemical alterations of preformed resins. Any crosslinked polymer will be termed a "resin" in this paper.

An ion exchange resin is a crosslinked polymer which contains either acidic or basic structural units and which can exchange either cations or anions on contact with a solution. Resins are insoluble in all solvents, due to their infinite molecular size which arises from the crosslinks. An excellent discussion of structure-property relationships in ion exchange resins is given by Bauman.[1]

The criterion of insolubility is an important one for most resin uses, and a resin which is crosslinked with 5 to 20 per cent of crosslinking agent (the range in commercial resins) is literally as soluble as a brick. Despite the enormous size of the resin molecules and their complete insolubility, such a polymeric acid or base will exhibit the chemical activity of a monomeric acid or base, subject to the restrictive conditions imposed by the insolubility and the immobility of the massive organic ion. These differences lead to complex thermodynamic relationships between the resin and a solution in contact with it. These relationships will be treated in succeeding papers of this monograph.

There are two general types of polymerization reactions, both of which can be used to synthesize ion exchange resins. These are known as condensation polymerization and addition polymerization. The formation of condensation polymers is accomplished by reacting polyfunctional molecules in such a manner that new carbon-carbon, carbon-nitrogen or carbon-oxygen bonds are formed. These act as the joining links which bind the starting molecules together into the polymeric network. Condensation reactions are always accompanied by the formation of one small molecule such as water, alcohol or ammonia, for each new carbon-carbon, carbon-nitrogen or carbon-oxygen bond created. Such polymers have a composition which differs from that of the monomers by the elements of the molecule which was split off in the polymerization. Some of the resins prepared in this fashion are not stable indefinitely over the entire pH range, as carbon-oxygen and carbon-nitrogen links are susceptible to hydrolytic cleavages.

In contrast, polymers prepared by addition polymerization of olefinic compounds contain only carbon-carbon bonds as the links which join the monomers. These polymers have the same empirical composition as the monomers used in their preparation. Such polymers are stable to heat and pH changes. An excellent bibliography of reported preparations of ion exchange resins up to 1950 is given by Kunin and Myers.[2]

FIGURE 1 shows the formation of a phenol-formaldehyde type resin by means of a condensation reaction between meta-phenolsulfonic acid and formaldehyde.[3] Each benzenoid ring in the product contains one sulfonic acid group which can exchange cations with a solution in contact with the resin. A high degree of crosslinkage, however, exists here, as each ring is joined (theoretically) to 3 other rings. This tight network creates a hard, brittle resin, and prevents the contacting solution from diffusing rapidly throughout the entire resin matrix. Indeed, the theoretical capacity is never reached, which is believed to be due to certain tightly enmeshed "pockets", inaccessible to the solvent. Such a resin will require a much longer time for exchange processes to reach equilibrium than for a soluble polymeric acid or monomeric acid. Simultaneously, the high degree of crosslinking will prevent extreme swelling or shrinkage of this type of resin with changing environment.

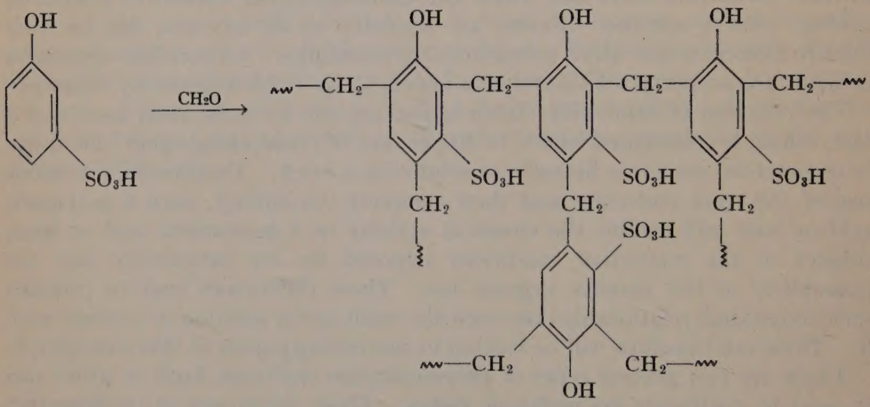


FIGURE 1

FIGURE 2 illustrates another phenol-formaldehyde type condensation polymer in which the sulfonic acid groups are attached to the alkyl side chains which link the rings together.[4] FIGURE 3 illustrates how a carboxylic acid resin and a sulfonic acid resin were prepared in Germany during World War II by condensation reactions.[5]

FIGURE 4 shows the preparation of a weak-base anion exchange resin by a condensation reaction.[6] A Mannich reaction is shown at the top. This reaction can occur at reactive positions in the phenolic rings of "Bis-Phenol-A", and competes with the usual formation of a methylene bridge between two rings. For simplicity the letter "R" is used to indicate the residue, enclosed in brackets, which arises from formaldehyde and tetraethylenepentamine. This particular resin was found to be effective as an antiacid agent[7] and either it or a related resin may be used in medicine for this purpose.

In FIGURE 5 is shown the preparation of a weak-base anion exchange resin by an amine-formaldehyde type condensation reaction.[8] Since there are so many possible ways in which the three reagents used can react, the structures shown are merely meant to represent possible structures which might exist in the resin. This is also true of all the formulas used to represent resins, as no absolute proof of the exact structure of any crosslinked polymer has ever been made, due to the inherent difficulties of dealing with a heterogeneous and insoluble resin.

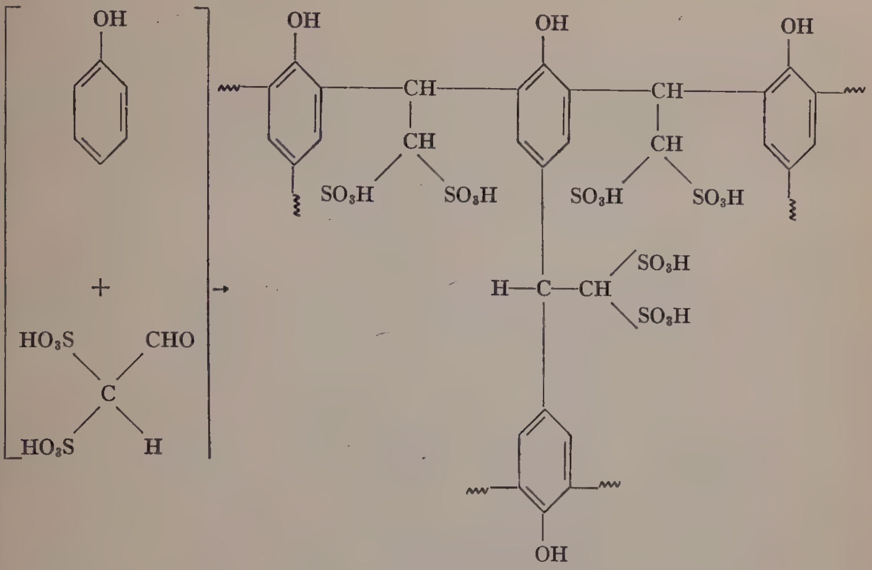


FIGURE 2

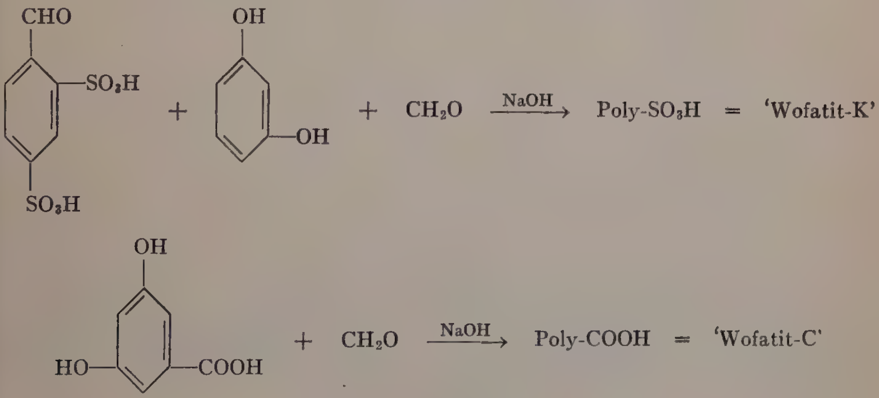
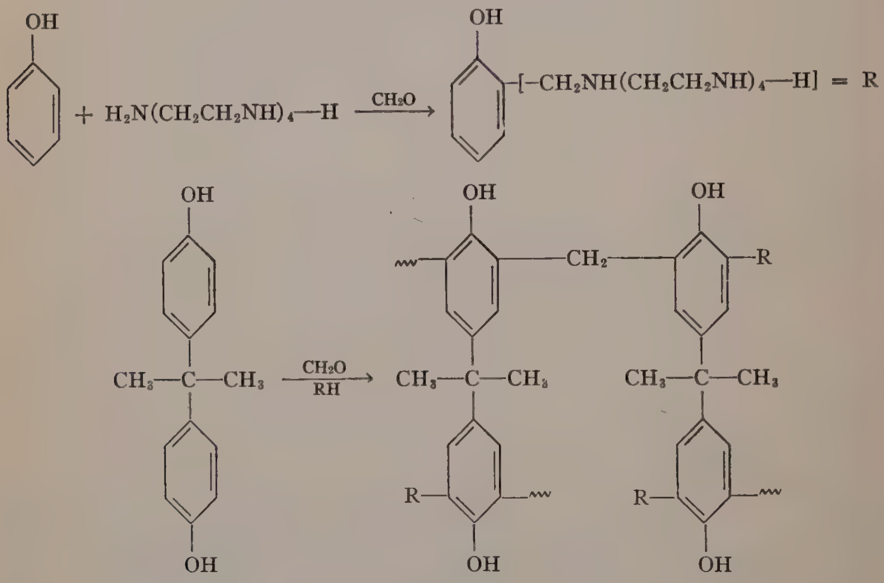


FIGURE 3

The structural features of several ion exchange materials which have been prepared from cellulose are shown in FIGURE 6.[9] The interesting feature of these exchangers is that some of them can be prepared from cellulose in bulk, yarn, or woven cloth without changing the physical form of the cellulose. Such materials are reported to exchange ions with great rapidity. Both weak and strong anion and cation exchangers have been made from cellulose.

Cation exchanging resins have been prepared from casein on treatment with formaldehyde.[10] These resins are susceptible to hydrolytic breakdown due both to the peptide nature of the backbone structure of the resin and to the C—N—C crosslinks. Capacities of such resins are quite low.

Some resins, which are prepared by addition polymerization methods and may be used directly as obtained, are illustrated in FIGURE 7. An advantage of addition polymerization over condensation methods in resin preparation is that the addition methods can be adapted to the preparation of the polymer in bulk, powdered or bead forms of various sizes. The condensation reactions usually give a bulk product which must be broken to desired sizes. Bulk polymers of acrylic or methacrylic acid, together with from 0 to 24 per cent of divinylbenzene as crosslinking agent, were studied by Bernstein and Gregor.[11] The total capacities were slightly reduced and the ability to swell was greatly reduced with increasing degree of crosslinking. The time required for establishment of equilibrium between a resin and the contacting solution varied directly with the degree of crosslinking.



"Bis-Phenol-A"

FIGURE 4

Similar polymers prepared using either divinylbenzene, butadiene or isoprene as crosslinking agents for methacrylic and acrylic acids were described by Bodamer.[12] The polymerization conditions used by Bodamer lead to the so-called "popcorn" resins, which are much less dense and more porous than the copolymers prepared by Bernstein. These resins exchange ions more rapidly than the bulk polymers. Even when the same ratio of starting materials is used, the "popcorn" resins are less dense than the bulk resins. Carboxylic acid cation exchange resins similar to those shown in FIGURE 7 have high total

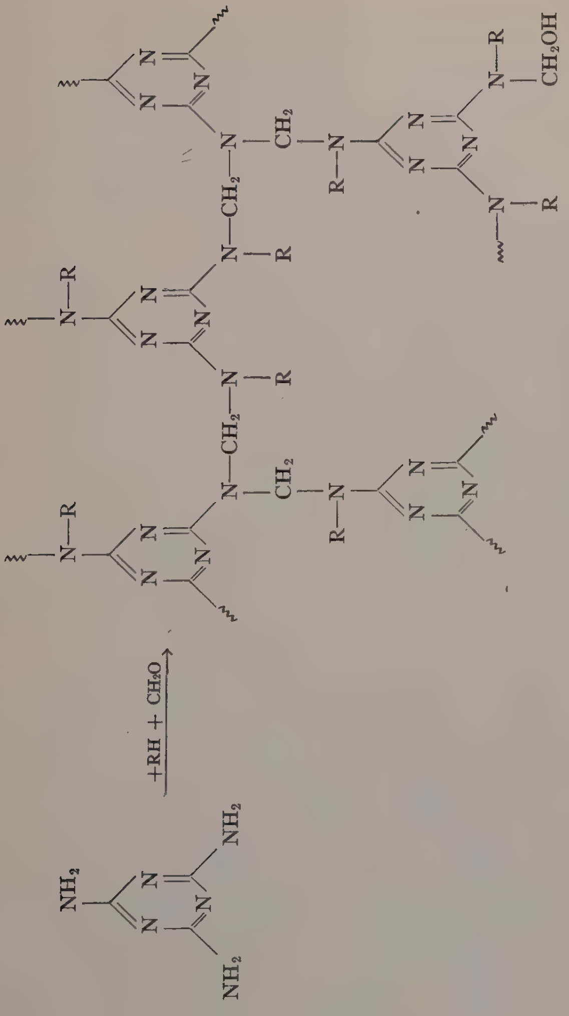


Figure 5

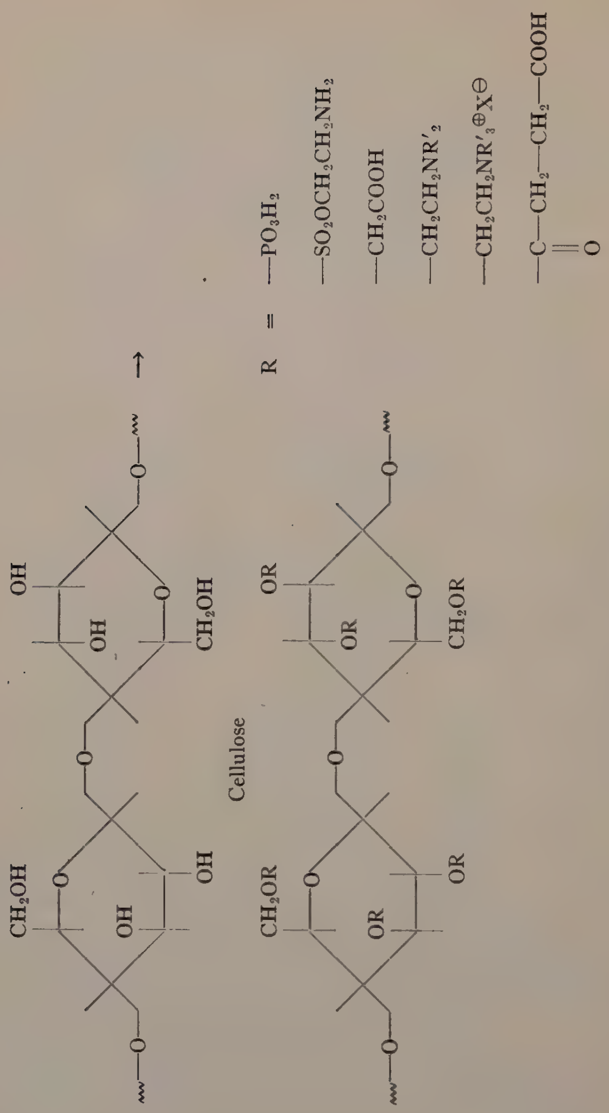
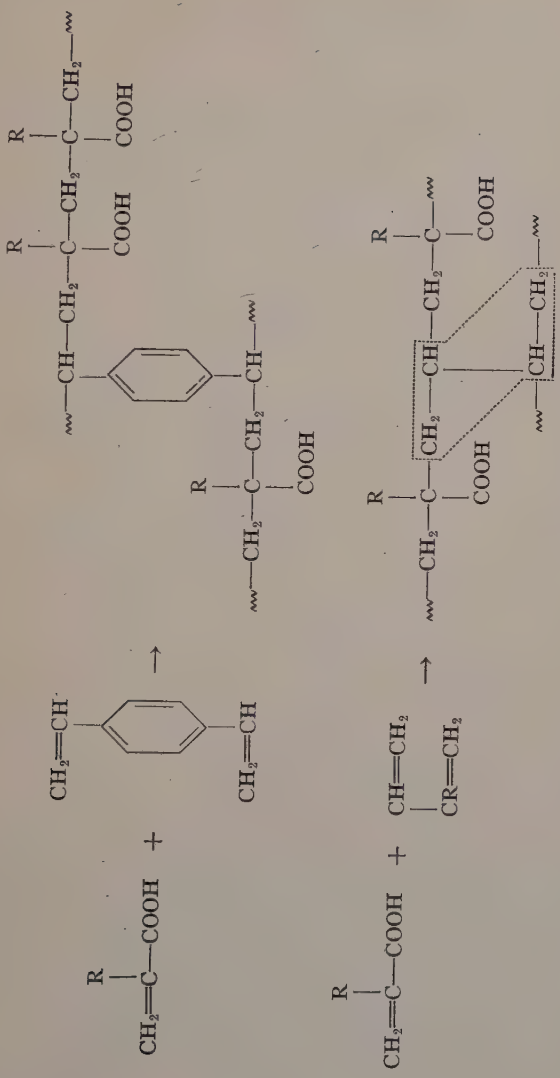

CH₂OH
OH
OH
OH
OH
CH₂OH
Cellulose
CH₂OR
OR
OR
OR
OR
CH₂OR
R =
—PO₃H₂
—SO₂OCH₂CH₂NH₂
—CH₂COOH
—CH₂CH₂NR'₂
—CH₂CH₂NR'₃⊕X⊖
—C(=O)—CH₂—CH₂—COOH

FIGURE 6



"Popcorn" Type Resins

FIGURE 7

capacities for cations, and are now being used in medicine to remove sodium ions from the human body upon ingestion.[13]

In FIGURE 8 is shown the basic structure of a styrene-divinylbenzene copolymer. Although the commercial divinylbenzene contains almost equal amounts of ethyl-vinylbenzene and divinylbenzene, we shall consider the basic structural unit to be that which is illustrated at the bottom of FIGURE 8. It is helpful to consider this basic structure to be analogous with that of benzene as a chemical intermediate.

Some reactions which can be applied to a crosslinked styrene polymer are shown in FIGURE 9. The sulfonation reaction leads to a polystyrene sulfonic acid in which it appears, from capacity determinations, that every benzene ring in the polymer contains one sulfonic acid group.[14] The resulting resin is a strong acid, and several commercial cation exchange resins have this general composition with about 10 per cent of crosslinking agent. Recently a series of such polysulfonic acid resins with various degrees of crosslinking has been made commercially available (Dow Chemical Company).

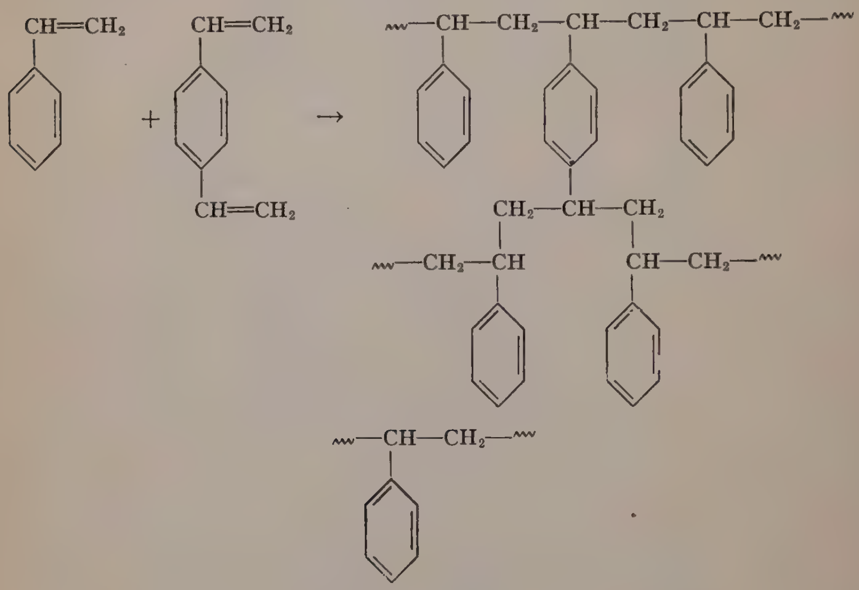

Repeating Unit

FIGURE 8

The chloromethylation of crosslinked polystyrene gives the intermediate resin, which is a polymeric benzylchloride. This can be reacted with secondary or tertiary amines to form either "weakly" or "strongly" basic anion exchange resins.[15] Analogous resins are commercially available at present. The chloromethylation of linear polystyrene is complicated by crosslinking reactions, and, unless the reaction is stopped before or at the introduction of chloromethyl groups into 65 per cent of the polymer, an insoluble product will form.[16] These results probably mean that in a crosslinked polymer, chloromethylation leads to further crosslinking. The anion exchange resins prepared by this route probably have an unknown degree of crosslinking in excess of that of the starting polymer. This factor has no bearing on practical uses of the resins.

The resulting quaternary ammonium resins have considerable industrial significance, as they can be used to remove weak acids, such as silicic and carbonic, from water. Weak amine resins can be prepared from the chloromethylated resin as illustrated.

In carrying out reactions upon these crosslinked resins, it is essential that the reactants can diffuse readily throughout the polymer matrix. The resulting polymer must be swelled by the reaction mixture to allow this diffusion, but it is not necessary that the initial resin be swelled by the reagents. For example, the crosslinked polystyrene resins are not swollen in sulfuric acid, but

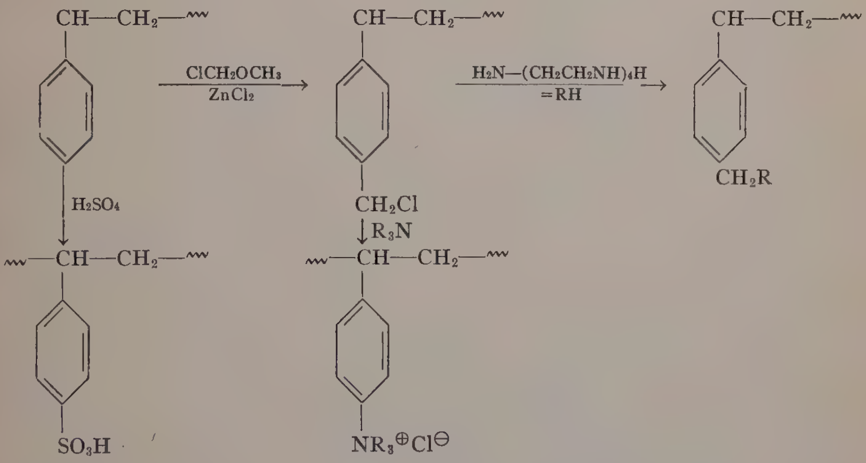


FIGURE 9

on heating the mixture with a catalyst the surface of the resin is sulfonated. The surface then consists of polystyrene sulfonic acid groups, which are swollen by sulfuric acid and allow it to diffuse into the interior until the entire resin particle is completely sulfonated and therefore has swelled to the limit allowed by the degree of crosslinking. Although it is not necessary for the polystyrene resin to be swollen before the sulfonation, it is advantageous to use an inert swelling agent such as trichlorethylene when bead particles are used.

Another example of the importance of the swelling factor is found when carrying out reactions with the chloromethylated resins. These are structurally analogous to benzyl chloride, and should readily hydrolize to the polymeric benzyl alcohol. The chloromethylated resin, however, is not swollen in water or alcohol, and beads of the chloromethylated resin which are preswollen by tetrahydrofuran shrink on addition of these solvents. Therefore, essentially no reaction occurs on heating beads of the chloromethyl resin with alcoholic potassium hydroxide solutions. This swelling factor is so important

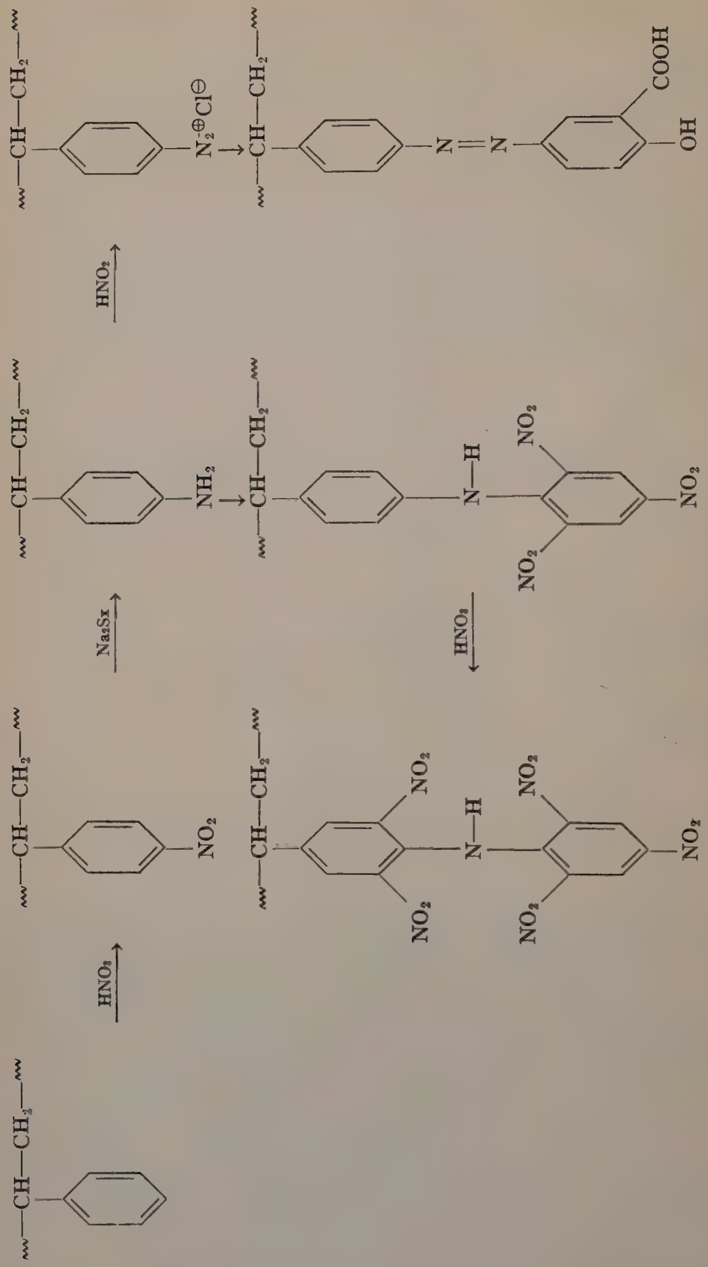


FIGURE 10

that if one observes that a resin is in a tightly-bound or unswollen state after being submitted to a set of reaction conditions, he may be quite certain that little or no reaction has occurred.

In the last few years, several groups have been interested in the preparation of ion exchange resins with a high degree of specificity for one ion. Gregor, *et al.*[17] have prepared a resin by the condensation of meta-phenylenediamine-diacetic acid with formaldehyde. This resin exhibits an unusual selectivity at different pH values which can best be attributed to the possibilities of chelate ion formation. It was found possible with this resin to adsorb cobalt from cobalt-iron-copper mixtures at a pH of 2.5, and to adsorb copper from the same mixture at a pH of 5.

Anders Skogseid,[18] in Norway, has accomplished the sequence of reactions shown in FIGURE 10, using linear polystyrene as his starting material. Nitration was accomplished with fuming nitric acid to give an almost quantitative yield of linear polynitrostyrene. Oxidative degradation of the polynitrostyrene gave only 4-nitrobenzoic acid. The reduction to the polyaminostyrene was accomplished with 60 to 80 per cent conversions by the use of sodium polysulfide and alcohol under pressure. The succeeding steps in the reaction sequence went well to give the polymeric analog of dipicrylamine. Dipicrylamine, itself, is a specific precipitant for potassium ions in the presence of sodium ions. The polymer also exhibits a high degree of specificity for potassium ions, and has been suggested as a possible agent for recovery of potassium from sea water.

The diazotization and coupling reactions carried out by Skogseid are shown to illustrate that it is possible to carry out quite an involved sequence of reactions upon linear polystyrene. Skogseid has prepared nine different resins by coupling with the diazotized polyaminostyrene, and has found some of them to be quite selective for potassium over calcium and magnesium and *vice versa.*

Recently, Craig, Hsia and Gregor[19] have successfully applied the nitration and reduction steps discussed above to divinylbenzene crosslinked polystyrene polymers in powdered or bead form containing from 1 to 10 per cent divinylbenzene. The nitration and reduction have been accomplished in overall conversions of 80 per cent, except for the 10 per cent crosslinked beads, which have been, as yet, only 30 per cent reduced. The use of a crosslinked matrix is desirable in such reactions, because the amount of swelling of the final products can be more carefully controlled than for the products obtained from linear polystyrene, which are assumed to be indefinitely and "accidentally" crosslinked. Diazotization has been accomplished using special conditions (to prevent shrinkage of the beads). Decomposition to the polyhydroxystyrene occurs on heating the diazonium salt in acid solution. Further study of the crosslinked resins is in progress.

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ELECTRON EXCHANGE POLYMERS: PRESENT STATUS OF THE PROBLEM*

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About twelve years ago, the Academy held a conference on free radicals at which Doctor M. Schubert[11] discussed the analogy between acid-base reactions and oxidation-reduction reactions. He pointed out that the differences observed in working with the two phenomena had caused their theoretical treatments to proceed along different lines despite the recognized analogies.[7, 12] Whatever the reason, it was not until 1949 that there was published what appears to be the first work in which this analogy was carried into the exchange-resin field.[3] While the analogy between acid-base and redox phenomena is strong when the two are considered as aspects of electrode reactions (as Schwarzenbach has shown[12]), the differences between the two phenomena emphasize themselves when the two are considered from the chemical standpoint of molecular reactions. In this report, we endeavor to show some of the analogies and differences as they have disclosed themselves and to summarize the present status of our work with electron exchange-polymers.

The concept upon which this work is based[3] is that, since it is possible reversibly to exchange protons at the surface of some cation exchange-resins, it should also be possible reversibly to exchange other fundamental particles, namely electrons, at the surface of some suitable polymeric substances. The exchange reaction in each case can be looked at as a distribution of the particular kind of particle between the resinous or polymeric mass and the ambient bulk fluid phase. This is the sense in which it might be considered a dynamic exchange, and is the sense in which electron exchangers bear an analogy to proton exchangers. When considered from the standpoint, however, that, with cation exchangers in the exchange process, protons are replaced in the resin by other cations it is seen that electron exchangers do not bear an exact analogy since the electrons are not replaced. They are lost, or gained, in the process (oxidation or reduction) along with a plus-charged ion, usually a proton. The proton exchangers are acid-base resins, the electron exchangers are redox resins.

When this work was initiated, a survey was made of possible redox systems suitable for incorporating in polymeric systems. Of the various well known reversible systems, the benzoquinone-hydroquinone one was chosen because so much is known about it. It was, of course, realized that the redox group chosen could be converted to a redox polymer by attaching it chemically (or even physically) to a natural or synthetic polymer which contained suitable reactive groups, but this approach was deferred in favor of the more general approach of synthesizing a polymer from suitable monomer.

It was decided to prepare vinylhydroquinone and polymerize this alone, or with other substances to form copolymers and terpolymers. Vinylhydroqui-

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none, a new compound, was prepared starting from coumarin.[13] When heated in a sealed tube under nitrogen to about 100° for a short time, it polymerized. The polymerized vinylhydroquinone, a white solid, was reported on earlier.[13] It was later found to be a low molecular substance. Analyses for functional groups and molecular weight determinations indicated largely dimer and trimer. Undoubtedly, the presence of the hydroquinone group in the monomer inhibited the chain-propagating reaction. That the oxidation behavior of the low molecular weight substance was to some extent duplicated by later work on high polymer is because the substance is relatively insoluble in aqueous solvents, thus providing a heterogeneous system, and because some of the titrations were carried out with the substance dispersed in the presence of bovine serum albumin, giving in effect a polymeric mass with adsorbed redox groups, also in a heterogeneous system.

High polymer was readily obtained when the hydroxyl groups of vinylhydroquinone were protected by preparing the diacetate or dibenzoate. After polymerization, the protecting groups are removed by saponification. Using this technique, polyvinylhydroquinone has been prepared, as well as copolymers of vinylhydroquinone and styrene, α-methylstyrene, 4-vinylpyridine and a copolymer of vinylhydroquinone and styrene crosslinked by divinylbenzene. The interest in copolymeric exchangers is twofold: first, to provide exchangers with properties such as acid or base solubility, for example, to permit their study and utilization under special conditions; secondly, to interpose other groups between adjacent redox groups so as to minimize interactions such as quinhydrone formation and thus enhance the availability of all the redox groups.

That we are indeed dealing with high polymers was shown by osmometric determination of molecular weight. The measurements were made by the static elevation method using a Zimm-Myerson osmometer.[14] A sample of polyvinylhydroquinone dibenzoate gave a molecular weight of 51,000, a copolymer of vinylhydroquinone dibenzoate and styrene gave a molecular weight of 41,000. These figures are not to be interpreted as indicating the highest molecular weights possible. The exchangers for which the molecular weight data were reported were prepared under polymerization conditions which would not be expected to lead to very high molecular weight polymers.

The procedure used in preparing these substances is in general as follows. The monomer, vinylhydroquinone, is prepared as stated previously from coumarin. It is a white crystalline substance melting at 111°. This is benzoylated or acetylated by the usual procedures using the acid anhydrides or chlorides. The benzoate is white and crystalline, melting at 94°. The white crystalline acetate melts at 48°. The monomer diester is mixed with the appropriate other monomer(s) such as styrene, α-methyl styrene, vinyl pyridine, in toluene, with a little benzoyl peroxide, *ca.* 0.1 mol per cent. The mixture in a glass tube is cooled in dry ice-acetone, evacuated and flushed with nitrogen several times, and sealed off at atmospheric pressure under nitrogen. It is polymerized by heat. After the reaction, the polymer can be purified by repeated dissolution in benzene or toluene followed by precipitation by pouring the solution into petroleum ether or methanol. The purified polymer is freeze-dried from benzene and is obtained as a white, porous solid.

Crosslinked polymer is prepared by polymerization of the mixture of monomers such as vinylhydroquinone dibenzoate and styrene (1 :1) with a small ratio of commercial divinyl benzene and 1 per cent by weight of benzoyl peroxide as small globules in a rapidly stirred aqueous medium containing surface-active agent and heated at 82° under nitrogen. This material is purified by washing with water.

The purified polymers in the ester form are converted to the free hydroquinone polymers by saponification with sodium ethylate in the strict absence of oxygen. Under alkaline conditions in the presence of oxygen, the polymers develop an intense blue color and, when isolated after such exposure, are obtained as red, insoluble substances, frequently resistant to reduction with hydrosulfite. When saponified, using apparatus which permits both the saponification and subsequent acidification to be carried out entirely in the absence of oxygen, the free hydroquinone polymers are obtained as white substances after freeze-drying from 90 per cent acetic acid or t-butyl alcohol containing a little water. These tend to turn pink slowly on exposure to air. That the treatment with sodium ethylate effects complete removal of ester groups is shown by the fact that correct saponification equivalents can be obtained and the resultant polymers can show the correct oxidation equivalents.

The linear hydroquinone polymers, such as polyvinylhydroquinone or the copolymers with styrene and α-methylstyrene, are insoluble in organic solvents such as glacial acetic acid, t-butyl alcohol, and dioxane. They become soluble very readily when a small percentage of water is added to the mixture of polymer and organic solvent. One possible explanation of this dependence of solubility on the presence of water is as follows. The polymer chains may be loosely crosslinked due to hydrogen bonds between the hydroxyl groups of hydroquinone units in different polymer chains. The effect of the water is to replace these loose hydrogen bonds with bonds between water molecules and hydroquinone hydroxyl groups, thus breaking down the crosslinking and permitting ready dissolution of the polymer in the organic solvent.

In a reducing medium, the hydroquinone form of the electron exchanger should predominate in the polymer and it should be possible to utilize the polymer to effect reduction of substances in a surrounding medium. In the process of effecting reduction, it gives up electrons to the substance being reduced and is converted to the quinone or oxidized form of the polymer. It should then be possible to utilize the exchanger to effect oxidation of substances in a surrounding medium, thus accepting electrons and being converted to the hydroquinoid or reduced form, which can then be used again for reduction purposes.

The electron exchange-polymers have been examined for redox behavior in three different ways: by electrometric titration; by testing the substances when dispersed on filter paper by the technique of radial chromatography (the crosslinked polymers cannot be tested this way unless paper is coated with the powdered polymer); and in column applications.[5, 6]

In electrometric titrations, if the polymer remains in solution or in colloidal form, and the titrations are carried out slowly, it is possible to obtain quite stable systems, with evidence of equilibrium at each step of addition of the titrating agent. Under such conditions, curves such as shown in FIGURE 1 are obtained. Note the increased slope and general linearity of the curve com-

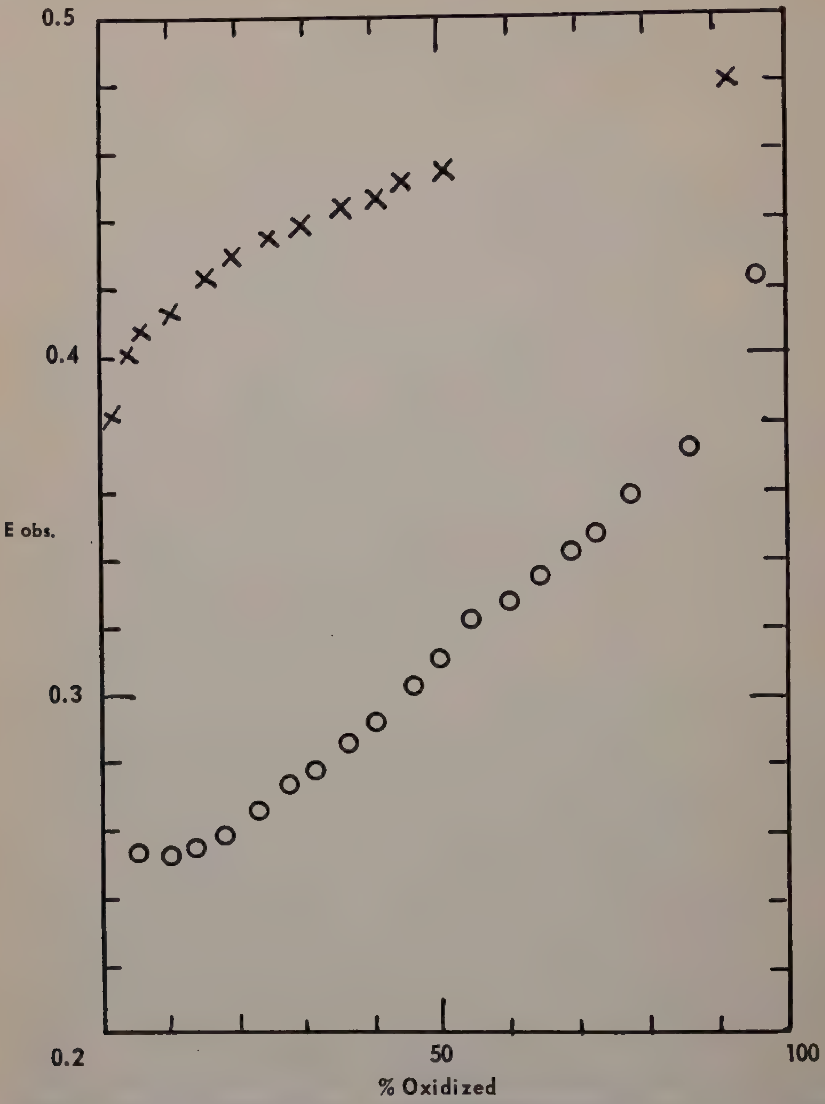

FIGURE 1. Lower curve: titration of 7.00 mg. polyvinylhydroquinone in 200 ml. 90 per cent acetic acid with bromine in 90 per cent acetic acid. The polymer was reduced overnight by bubbling with hydrogen in the presence of colloidal palladium.

Upper curve: titration of 5.245 mg. hydroquinone in 200 ml. 90 per cent acetic acid with bromine in 90 per cent acetic acid. The potentials were not very stable.

pared to hydroquinone. This may be due to the following considerations. It may be assumed that the spatial arrangement of an oxidizable unit in the polymer relative to other units may effect its ease of oxidation through interactions such as might arise from hydrogen bonding, dimerization or purely steric factors. If this is correct, then it follows that over these polymer chains there would be a spread of units with different oxidizabilities and, during a titration, the more easily oxidizable ones would go first, with those groups which oxidize with greater difficulty requiring a higher potential. The titration curve would then be "smeared out" over a wider potential range than for a single substance. This view is supported by the observation that a mixture of hydroquinone and ethylhydroquinone gives a titration curve which differs from that for hydroquinone in the same way as does the curve for polyvinylhydroquinone. The idea that many groups of slightly different potential are present is also supported by analogy to the titration behavior of polycarboxylic acids, where a spread of dissociabilities of the protons would be expected, and it is known that the curve for polyacrylic acid is different than that for isobutyric acid.[11]

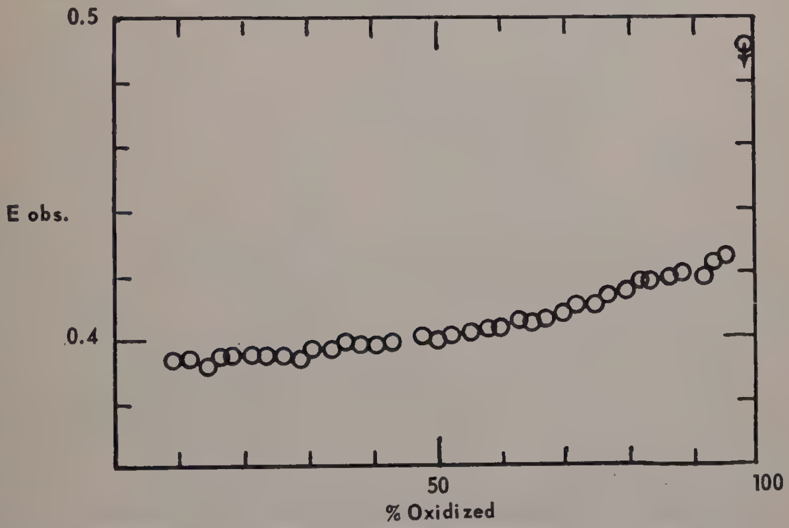

FIGURE 2. Titration of 14.765 mg. copolymer of vinylhydroquinone with α-methyl styrene in 200 ml. 90 per cent acetic acid with bromine in 90 per cent acetic acid. The potentials were reasonably stable except for that with a small arrow, which drifted slowly downward.

If the polymer precipitates during the titration, stable potentials can also be obtained, but the data disclose the flattened curves (FIGURE 2) associated with such a phenomenon,[9] and believed to be due to the failure of the oxidized groups to accumulate effectively as a result of the precipitation.

In either case, whether precipitation does or does not occur, the solutions take on a pink tinge, as the titration progresses, which decreases in intensity after the end point has been passed, and frequently gives place to a yellow color in the oxidized, usually insoluble, substance. Attempts to reduce the oxidized polymer in the customary manner of bubbling with hydrogen in the presence of colloidal palladium have not led to complete reduction of the polymer, presumably, because of the insoluble nature of the oxidized form.

It should be pointed out that in working with these polymeric substances, under conditions of considerable dilution, and with every likelihood and frequent visual evidence that the particle size changes during the reaction, it is often not possible to obtain two electrodes which show the same potential in a given system, or which come to a stable value. Despite these difficulties, experience with many kinds of behavior leads to the conclusion that the polymers do show redox properties and that the potentials exhibited are of the right order of magnitude, and show the proper pH dependence. Nevertheless, because of the uncertainties about junction potentials, *etc.*, the data presented in FIGURES 1 and 2 are not corrected to the normal potentials.

The redox polymer may be dissolved in a small amount of solvent and used to impregnate filter paper. Such paper can then be utilized as a *two-dimensional exchanger*. The paper is placed between two glass plates, the upper of which is provided with a small, centrally located hole through which reagents can be introduced. If the polymer is in the reduced form, a drop of dilute iodine-KI solution, introduced into the paper and washed in with neutral buffer loses its brown color (it forms a dark zone at first on the paper) and, on removing the upper glass plate and spot- or streak-testing with starch, it shows no trace of iodine. There is present a zone of oxidized polymer, however. If the iodide is washed out of the central zone with a few drops of neutral buffer, then a few drops of dilute acid are applied. There is no reaction until a drop of acid KI solution is introduced, when there is immediate formation of iodine (yellow color, starch test) in the region of the initial zone. By the use of such a technique, the reducing power of the paper to ferric ions and to ferricyanide can be readily demonstrated. It is to be expected that this kind of test paper may prove to have many uses.

If the linear type of polymer is dissolved and precipitated onto a diatomaceous filter aid, which spreads it out and gives a bulkier mass for easier handling, it can be packed in a chromatography tube to form a *redox* or *electron-exchanger column.* Packing is conveniently done by sedimentation onto a layer of purified filter aid. The crosslinked polymer can be utilized in a column by first triturating it with filter aid and then sedimenting this mass to form a column. When more hydrophilic, less highly crosslinked polymers are prepared such treatment will be unnecessary.

In either case, the redox column can be utilized to reduce or oxidize substances depending on its state. For example,[5, 6] a column containing the vinylhydroquinone-α-methyl styrene copolymer, and calculated to have a capacity of 0.117 milliequivalent of reducing power showed the behavior indicated in TABLE 1 when treated alternately with oxidizing and reducing agents with intermediate washing of all reagent from the column. The column itself was *ca.* 1.5 cm. high and 1.3 cm. in diameter; the flow-rate was *ca.* 5 ml/30 min.; but could be readily increased by applying slight air pressure.

It can be seen from TABLE 1 that not only are the reactions of the polymer reversible, but quite good checks can be obtained. To the freshly packed column, when excess iodine was added in a solution containing potassium iodide at a pH of *ca.* 7.8; 0.122 meq. of iodine was reduced. The column was thus converted to the oxidized form of the exchanger, and its oxidizing power was demonstrated by its oxidation of iodide ion in acid medium to free iodine to the extent of 0.117 meq. The exchanger was thus converted to the reduced form. The agreement between 0.122 and 0.117 meq. is reasonable, considering that the effluent was in each case collected in fractions (7 and 8 respectively) and titration errors probably accumulated. The redox cycle was then repeated, with the reduction of 0.105 meq. of iodine and then the oxidization of 0.098 meq. of iodide ion. Agreement in this cycle is also reasonable. The lower values can be accounted for by channelling in the column, or by not having a sufficient excess of reagent. The column then reduced 0.106 meq. of dichromate ion, after which it oxidized 0.122 meq. of iodide ion to iodine. The column then remained under dilute acid potassium iodide for two and a half days, after which it reduced 0.153 meq. of dichromate. The analysis for dichromate may have been faulty. The loss in capacity which ensued later, however, is not an artifact. It can be seen from the data at the lower end of the table that the capacity of the column was greatly decreased, possibly irreversibly. This may result from a gathering together of the initially spread out molecules under the influences of the changes in pH and potential in the repeated cycles. It should be possible to correct it by known methods, such as proper crosslinking.

TABLE 1

BEHAVIOR OF AN ELECTRON-EXCHANGE COLUMN

Treatment	Corresponding millequivalents
Fresh column	0.117
Excess iodine in KI at a pH of *ca.* 7.8. Iodine was reduced	0.122
Excess acid KI. Iodide was oxidized to iodine	0.117
Excess iodine in KI at a pH of *ca.* 7.8. Iodine was reduced	0.105
Excess acid KI. Iodide was oxidized to iodine	0.098
Excess dilute acid dichromate. Dichromate was reduced	0.106
Excess acid KI. Iodide was oxidized to iodine	0.122
Excess dilute acid dichromate. Dichromate was reduced*	0.153
Excess acidified Mohr's salt. Ferrous iron was oxidized	0.019
Excess dilute ferric chloride. No detectable reduction	0.000
Excess acid KI. Iodide was oxidized	0.019
Excess cystine in alkaline medium. Doubtful reduction	0.009
Excess iodine in KI at a pH of *ca.* 7.8. Iodine was reduced	0.028
Excess acid KI. Iodide was oxidized to iodine	0.032

* The column had remained under acid KI for 2½ days before this experiment. The analysis for dichromate may have been faulty.

The countercurrent application of redox polymers has certain features which deserve emphasis, especially in contrast to batchwise applications. In this respect, the differences in the two methods of application show clear analogy to those in the ion exchange field. Thus, the batchwise application, stirring the resin into the solution to be treated, until a definite potential is reached, would be used to *poise* a system. The column technique would not be satisfactory here. On the other hand, the column technique, because of its differential countercurrent nature, makes the most efficient use of the exchanger in any given reaction. Indeed, it might be expected that the polymer would reduce a substance the normal potential of which is close to that of the polymer. The moving substance passing down the column always comes into contact with more reduced form of the polymer and thus is effectively exposed to a lower potential than if it were just mixed with the polymer and the process allowed to go to completion. A further, most important, feature of the use of these substances lies in the fact that, since they are quite insoluble in aqueous solvents, they do not contaminate the solutions with which they react unless the contribution of protons (in the reduction cycle) is considered a contamination.

It appears, then, that we have in these substances tools of considerable importance for use in oxidation or reduction processes. The operating potential of these resins is pH-dependent, which gives a measure of control over it. Another method of adjustment can be visualized. The chemical method of using either different redox groups instead of unsubstituted hydroquinone or of further substituting the ring with other rings, or with other functional groups which raise or lower the potential by inductive, resonance, and such, effects. It should thus be possible to run the gamut of oxidation potentials. It is conceivable that, by proper selection of an exchanger, it may be possible to oxidize or reduce very selectively, without contamination, certain components of a mixture and leave untouched others which might require an exchanger of a different potential.

Redox resins should find wide application to biochemical problems as a means of controlling and studying the effect of redox potentials in many systems without causing contamination. Industrial applications should also be possible, both for preparative purposes and as a means of facilitating removal of impurities.

Obviously, there is scope in this field for many workers and many new ideas. A good deal of scientific progress, perhaps the major part, is based on analogy, exact or loose. In this field of electron exchange-polymers the similarities to ion exchange-polymers as well as the differences can surely be exploited with much profit to science.

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THERMODYNAMIC PROPERTIES OF ION EXCHANGE RESINS; FREE ENERGY OF SWELLING AS RELATED TO ION SELECTIVITIES*

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Ions exchange resins are essentially crosslinked polyelectrolyte systems, having physical properties which are similar to those of the soluble polyelectrolytes in most respects, but being subject to the restraint of limited swelling as a result of crosslinking.[1, 2, 3, 4] Since these are Donnan systems because the fixed ionic groups are restricted to the resin phase, Gibbs-Donnan theory can be applied. This paper describes the swelling properties of ion exchange resin systems as determined by the crosslinking, the nature of the exchange ion, and the interaction, if any, between the fixed groups and the movable exchange ions.

Resin Model Systems

The system itself is shown in FIGURE 1. Here a sulfonated polystyrene-divinylbenzene (DVB) cation exchange resin is depicted. The polyelectrolyte is shown swollen with water, so that the exchange cations are dissociated and free to diffuse and to exchange. The resin shown has about 8–10 per cent crosslinking and, in the water-wetted state, is about 6 molal with respect to the fixed groups.[5]

The movable, and thus osmotically active exchange ions within the resin phase, lower the activity of the solvent causing more solvent to enter until the swelling pressure within the resin is counterbalanced by the elastic tension of the matrix. Two expressions apply.

The first, shown in its simplified form, is for the thermodynamic osmotic pressure

$$\pi = \frac{RT}{\bar{V}_w} \ln \frac{a_w^o}{a_w^i} \cong \left(\frac{n^i RT}{V - V_M - b} \right) (a_w^o \cong 1),$$

where a_w is the activity of the water in the resin i and in the external solution phase o; n_i is the number of moles of osmotically active ions in the resin; V the volume of the resin phase; V_M the matrix volume and b is the effective (hydrated) volume of the exchange ions. The term b is then analogous to that used in the van der Waals equation of state for the effective molecular volume. This expression, then, shows that for a given resin system, π is proportional to the concentration of movable exchange cations and thus will increase with the exchange capacity of the resin and will decrease if the exchanging cations become associated with the fixed anions. Also, π will increase with b or as the size of the exchange cations increase.

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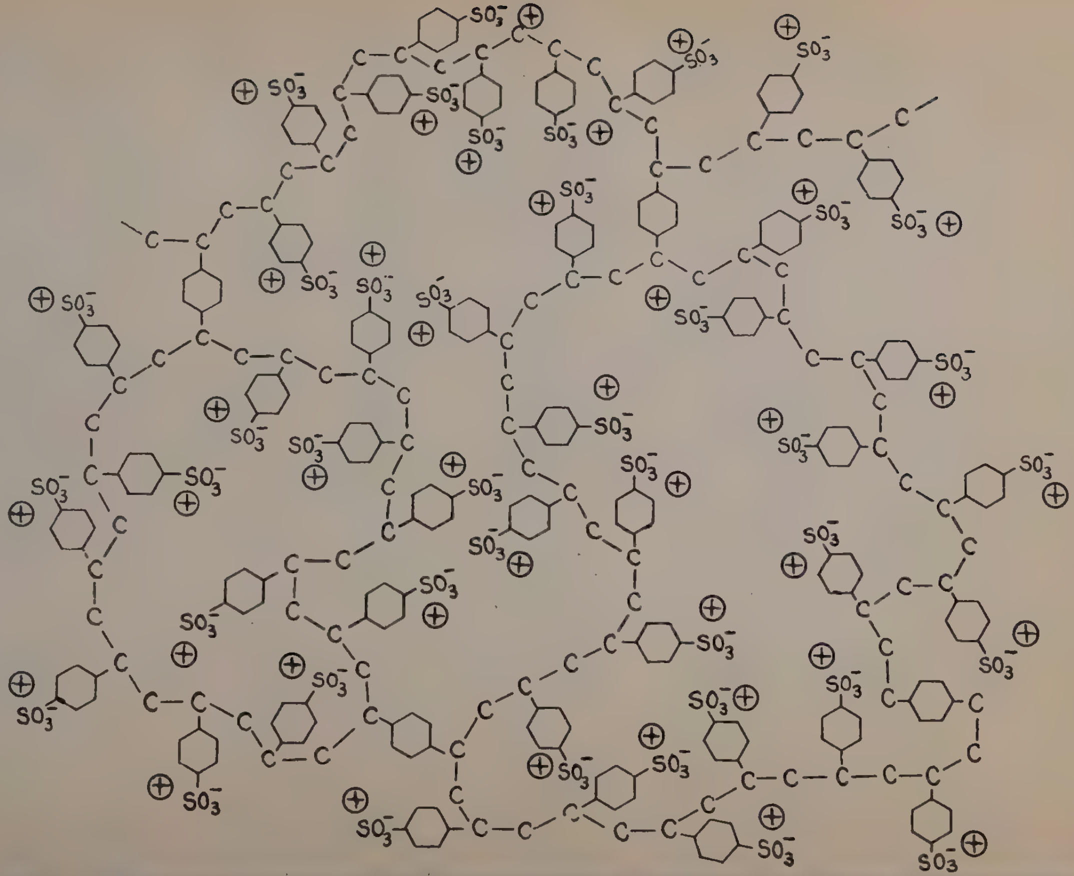

FIGURE 1. Structural diagram of polystyrene-sulfonic aciddivinylbenzene cation exchange resin in the swollen state. Exchange cations are shown ⊕, the solvent is not shown.

The second, simplified expression is for the elastic modulus of the matrix, and is

$$V = m\pi + V_o\,,$$

where V_o is the "rest" volume of the resin, corresponding to the state where π is zero, and m is the elastic constant.

Both of the above expressions are of course quite approximate. First, it must be remembered that the free energy of sorption of water, corresponding to the transport of a mole of water from the external solution where its activity is a_w^o to the resin at a_w^i is

$$\Delta F = RT \ln \frac{a_w^i}{a_w^o}$$

and is well defined thermodynamically. The corresponding osmotic work $\pi\bar{V}_w$ has validity only in terms of this free energy of sorption. The use of a thermodynamic osmotic pressure term does not necessarily imply that an actual hydrostatic pressure is present within these systems. If the resin gel were enclosed by a water permeable membrane and allowed to sorb water, then a true osmotic pressure would be exerted, which would be equal to the swelling pressure of the gel. As has been pointed out by several authors, particularly Alfrey,[6] the free energies of swelling and deswelling are best treated from entropy considerations, although it is convenient to calculate them in terms of osmotic pressures and volume changes.

The expression for π is approximate, in that effects of Debye-Hückel type are not considered. The use of a volume correction term similar to some of the corrections applied in the Bjerrum-Harned-Stokes and Robinson theory,[7] however, yields a good approximation in highly concentrated solutions. The assumed linearity between the volume of the resin phase and π is also approximate.[2]

The swelling properties of the model can now be calculated. Consider first the swelling of a specific resin system on the sorption of water at different relative humidities (p/p^o). The numerical values for the various parameters are taken from data for polystyrene sulfonic acid resins. The value for the parameter V_M can be taken to be the dry volume of the resin, approximately 150 ml. per mole of fixed groups.[5] By a simple process of curve fitting, as will be described later, values of the other parameters are taken as follows: $m = 2$ ml. atm.$^{-1}$, $V_o = 300$ ml. for a rather weakly crosslinked resin, DVB 6; $m = 1$ ml. atm.$^{-1}$, $V_o = 200$ ml. for DVB 10, with average crosslinking; $m = 0.75$ ml. atm.$^{-1}$, $V_o = 125$ ml. for DVB 17, with higher crosslinking; $m = 0.5$ ml. atm.$^{-1}$, $V_o = 50$ ml. for DVB 23, with high crosslinking. The ion volume b is taken to be zero for these calculations. The sorption of water at different relative humidities can now be calculated. FIGURE 2 shows the moles of water sorbed at different relative humidities by resins varying from soft gels to hard, dense materials. The restraint of the crosslinking upon swelling makes itself felt at higher values of p/p^o, when the non-crosslinked material swells to infinity in accordance with Raoult's law.

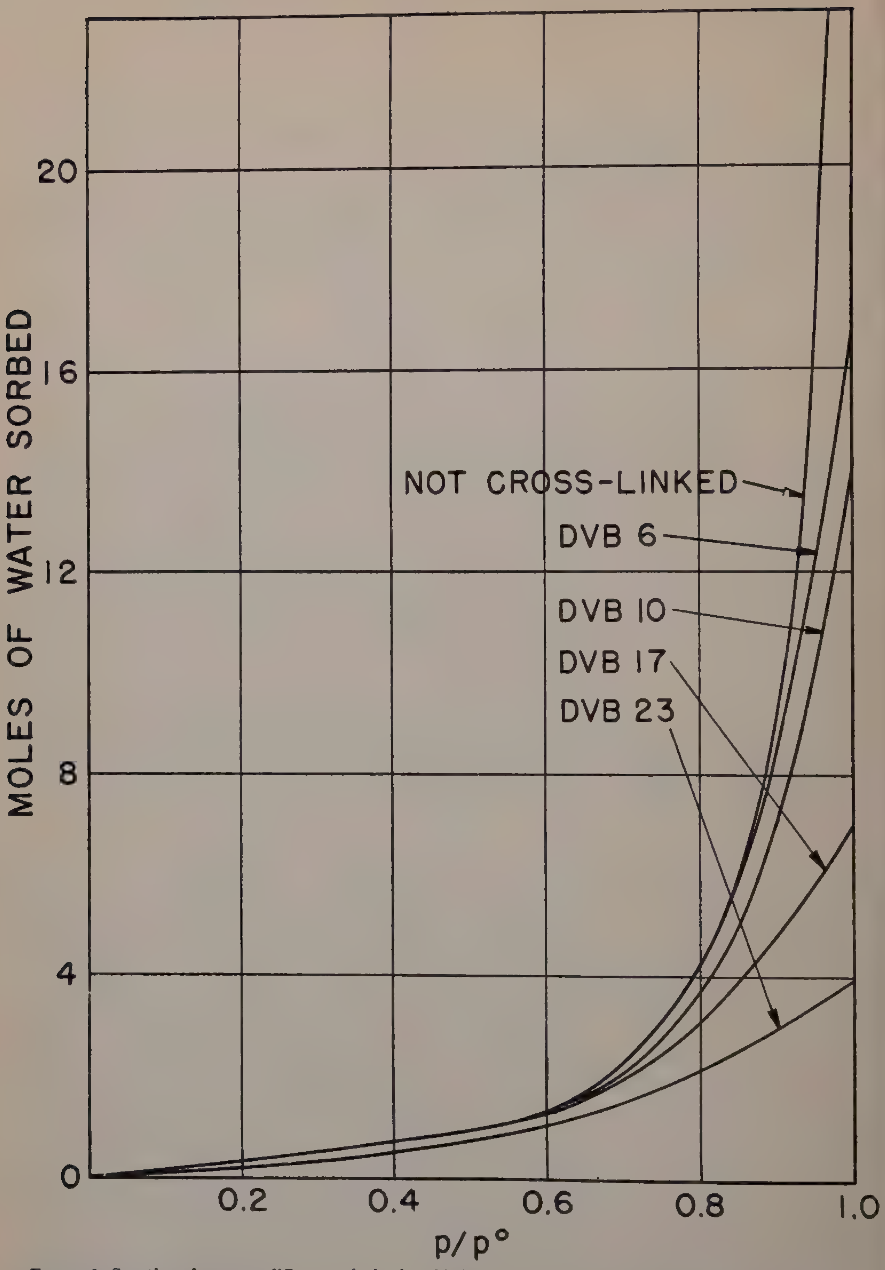

FIGURE 2. Sorption of water at different relative humidities, calculated for (ideal) model ion exchange systems of varying degrees of crosslinking.

For an ideal system as shown in FIGURE 2, the thermodynamic osmotic pressure and free energy of swelling can be calculated from the data by taking as a base line the sorption curve for non-crosslinked or slightly crosslinked material where $\pi \cong 0$, drawing a horizontal line corresponding to systems of the same composition, and calculating the osmotic pressure for the crosslinked resin from the relationship,

$$\pi = \frac{RT}{\bar{V}_w} \ln \frac{p_x}{p_1},$$

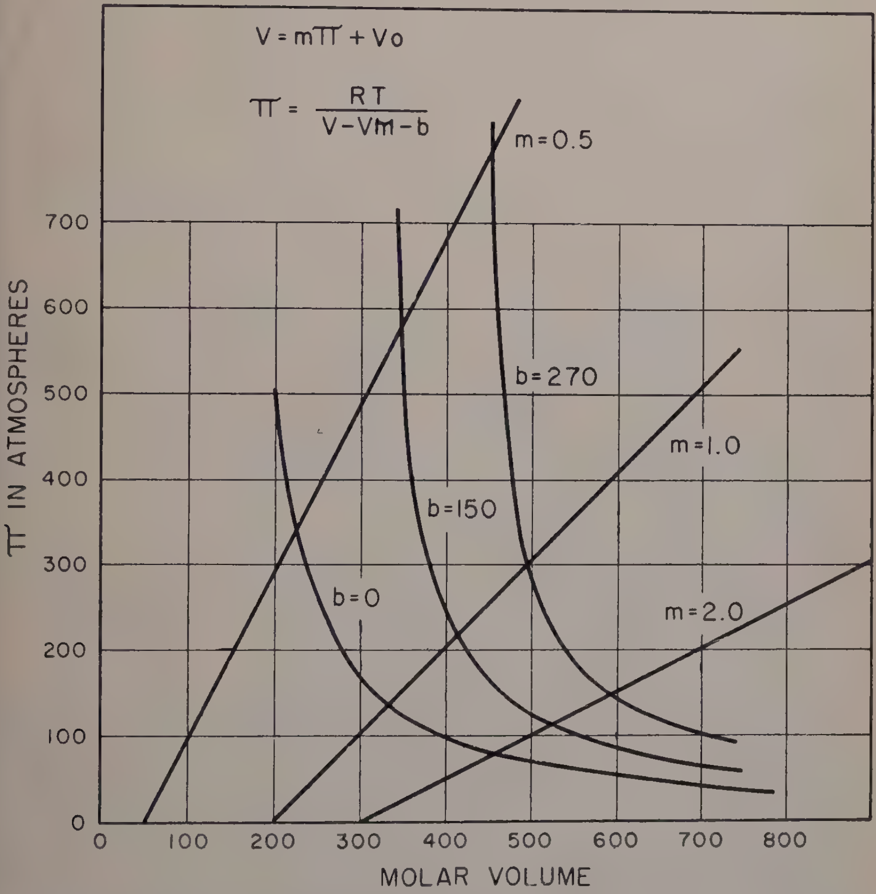

FIGURE 3. Calculated curves showing pressure-volume relationships describing the elastic tension of the resin matrix (linear) and the osmotic swelling pressure (hyperbolas).

where p_x and p_1 are the vapor pressures of water for the crosslinked and the linear polymers respectively. This method, suggested by Gregor,[2] will be applied to data for real systems in a later section.

For ion exchange resin systems in equilibrium with dilute aqueous solutions ($a_w \cong 1$), the swelling of the various model resins with different ions in the exchange positions can be calculated. The final, swelled volume corresponds to the intersection of the linear elastic modulus function which describes restrictions on the swelling, and the hyperbolic osmotic pressure function for the swelling pressure itself. A plot of these two functions, using the parameters given previously, is shown in FIGURE 3. Here it is seen that for a given resin, the resin volume increases with the change in volume b, and that, for the same exchange cation, the resin volume decreases and π increases as the per cent crosslinking increases.

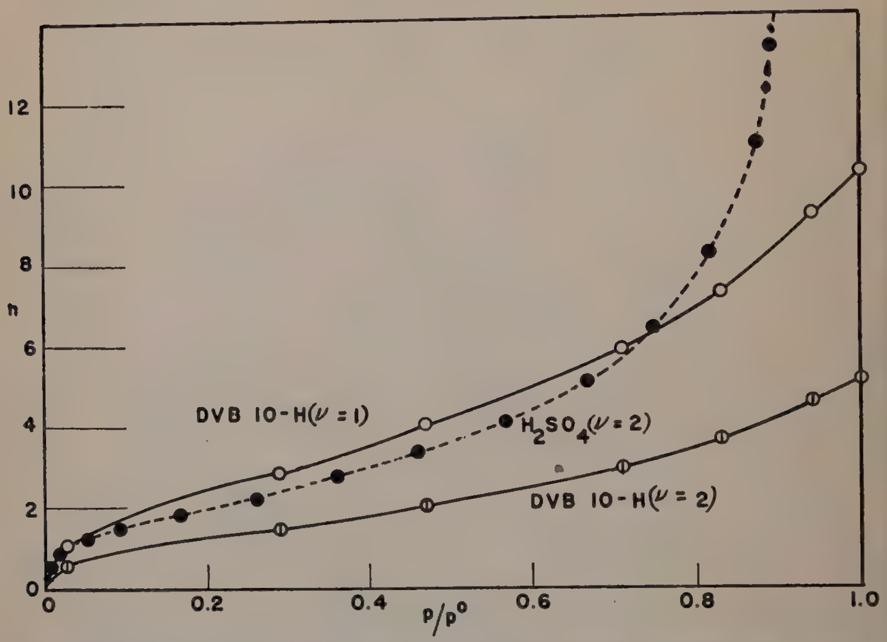

FIGURE 4. Moles of water sorbed per half-mole of sulfuric acid (●), per mole of exchange groups of a 10 per cent crosslinked polystyrene sulfonic acid resin (○), and per half-mole of exchange groups (⦶).

Real Systems—Sorption of Water

The sorption of water by ion exchange resin systems has been studied by Gregor, Sundheim, Held and Waxman,[4] and also by Glueckauf and Duncan.[8] The sorption of water by a typical sulfonated polystyrene –10 per cent DVB resin is shown in FIGURE 4, and is compared with data for a corresponding monomeric acid. The data is plotted as moles of water sorbed per mole of exchange groups. For sulfuric acid, the data is plotted in terms of two osmotically active groups per molecule ($\nu = 2$), namely the H^+ and the HSO_4^- ions. The secondary dissociation of sulfuric acid does not become appreciable until fairly dilute solutions are attained. The sharp rise of the sulfuric acid curve at high values of p/p^o is due partly to the secondary dissociation, partly to Raoult's law effects. When the resin data is plotted in terms of one osmotically active ion for each mole of exchange capacity ($\nu = 1$), the close fit to the sulfuric acid data indicates that the H^+ ion is active, while the fixed sulfonate group is not. This is emphasized by the lack of fit when a $\nu = 2$ plot is made.

FIGURE 4 indicates the electrolytic character of the resins, as evidenced by the similarity of the sorption curves for the two acids. This similarity suggests that hydration effects and interionic forces are of about equal magnitude for both systems, and that principal differences arise because the resin matrix restrains the swelling of the polyelectrolyte acid. The data of FIGURE 4 may be compared with the calculated curves shown in FIGURE 2. The sharp, initial rise in sorption at low relative humidities is primarily the result of hydration of the hydrogen and sulfonate ions. Once this primary hydration has taken place, the sorption rises smoothly with increasing values of p/p^o.

The sorption of water by resins of varying degrees of crosslinking and in the hydrogen state is shown in FIGURE 5.[9] These results are at first view somewhat surprising. The more highly crosslinked resins DVB 8 to 23 all show substantially the same sorption at low relative humidities and, at higher relative humidities, sorb relatively more water as the crosslinking decreases. The loosely crosslinked resin (DVB 2) sorbs slightly less water than the higher crosslinked resins at low relative humidities, but crosses over the curve for DVB 8 at higher relative humidities. At very low degrees of crosslinking (DVB 0.4), the sorption is markedly depressed at low relative humidities, but as more water is sorbed the curve crosses over and becomes substantially the same as for DVB 2. The sorption curve of the linear polymer is most striking. It is very strongly depressed at low relative humidities, rises very slowly and, finally, approaches the curves for DVB 0.4 and 2 at molalities of about 5.

This behavior has been interpreted as being the result of configurational entropy effects by Sundheim, Waxman and Gregor,[9] who have calculated from data the free energies, heats and entropies of these sorption processes. Consider three of the polymer systems shown in FIGURE 5 at points where all have the same composition, *i.e.* along a horizontal line where the same amount of water is sorbed (same values of n). For example, at $n = 2$, the resin systems DVB 2 to 23 have activities of water of about 0.2, for DVB 0.4 the activity is 0.27, and for the linear polymer it is 0.41. This means that, while all of these systems are at the same concentrations with respect to exchange groups, the water in the linear polymer system exerts a vapor pressure twice that for the crosslinked resins. This result may be interpreted by saying that the solvent in the linear material is "free" relative to that which is "bound" in the resin systems. FIGURE 6 shows diagrammatic sketches of four different polymer-solvent systems. FIGURE 6a shows a polymer having an average degree of crosslinking. The polymer chains are shown in two dimensions, with four points of attachment. The density of lines per unit area is then proportional to the concentration of the polymer, and the average distance between neighboring polymer chains indicates the configurational entropy. A more highly crosslinked polymer is shown in FIGURE 6b, where the degree of crosslinking is 2.5 times that of 6a, but where the configuration is approximately the same as for 6a so that polymer-solvent and polymer-polymer interactions are equal. An ideal linear polymer system having about the same configuration as the crosslinked polymers is shown in FIGURE 6c. Since all of these systems (a, b,c) have the same configurational entropy, then their thermodynamic osmotic pressures could be calculated directly from their solvent activities, as shown previously. The real linear polymer system, however, shows thermodynamic properties which indicate that its configurational entropy is as shown in FIGURE 6d, where the polymer chains are not uniformly separated from one another, but where they are bunched together, held by van der Waals's forces. This strong increase in polymer-polymer interactions means a decrease in polymer-solvent interactions, and the solvent activity is correspondingly increased.

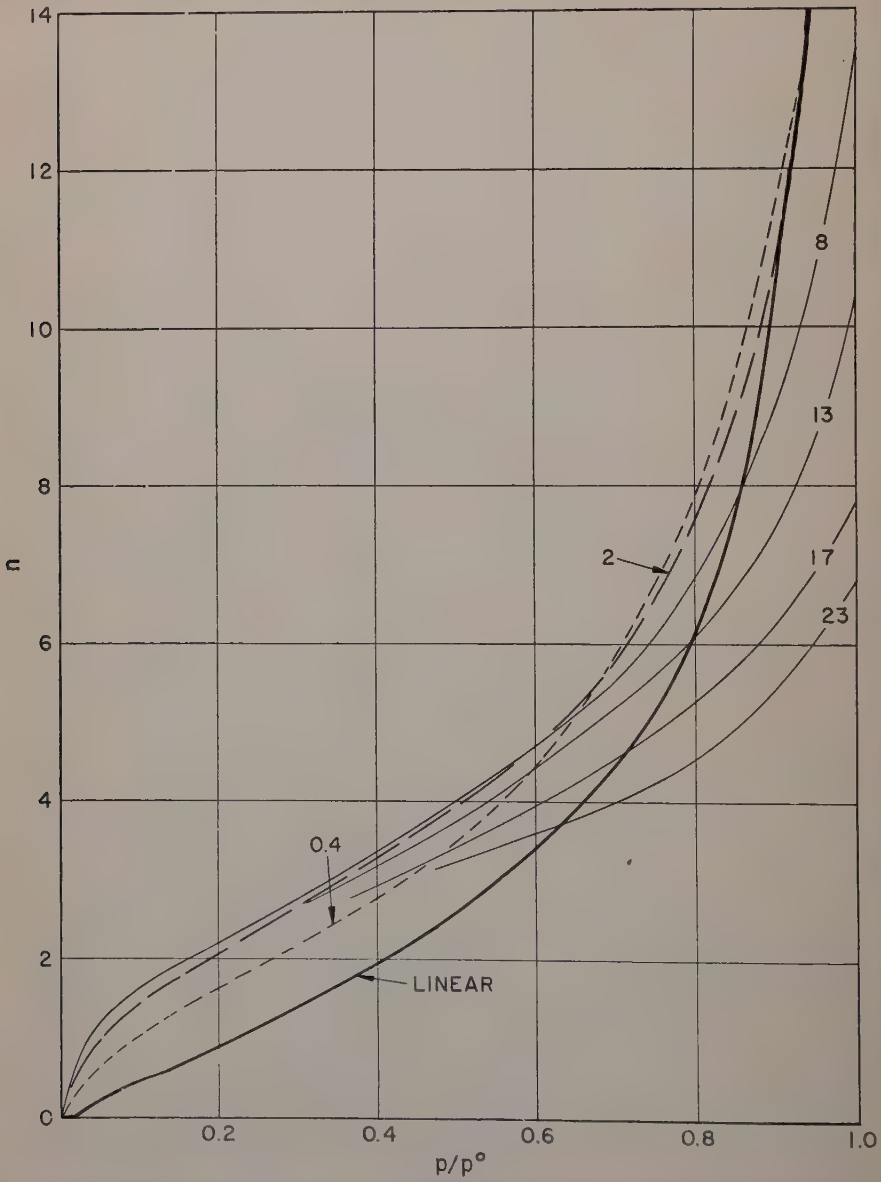

FIGURE 5. Moles of water sorbed per mole of exchange groups for polystyrene sulfonic acid resins of varying degrees of crosslinking at 25°C.

Calculation of Osmotic Pressures

An examination of FIGURE 5 thus shows that, while it might be preferable to base calculations of osmotic pressures upon data from linear polymer systems where it is known that π is zero, this is not practical. For example, where the linear sorption curve is to the right of that for a crosslinked resin, a *negative* value of π would be calculated. An examination of FIGURE 5, however, also shows that, while at low values of p/p^o these anomalies exist, at higher values the curve for the linear polymer coincides with those for DVB 0.4 and 2. It may be concluded that at these dilutions polymer-polymer interactions are small in all cases, and also that π is virtually zero for both DVB 0.4 and 2. More important, FIGURE 5 shows that the curve for the very loosely crosslinked resin DVB 2 falls into the regular sequence and, therefore, has a configurational entropy comparable to that of the other, more highly crosslinked resins. Since

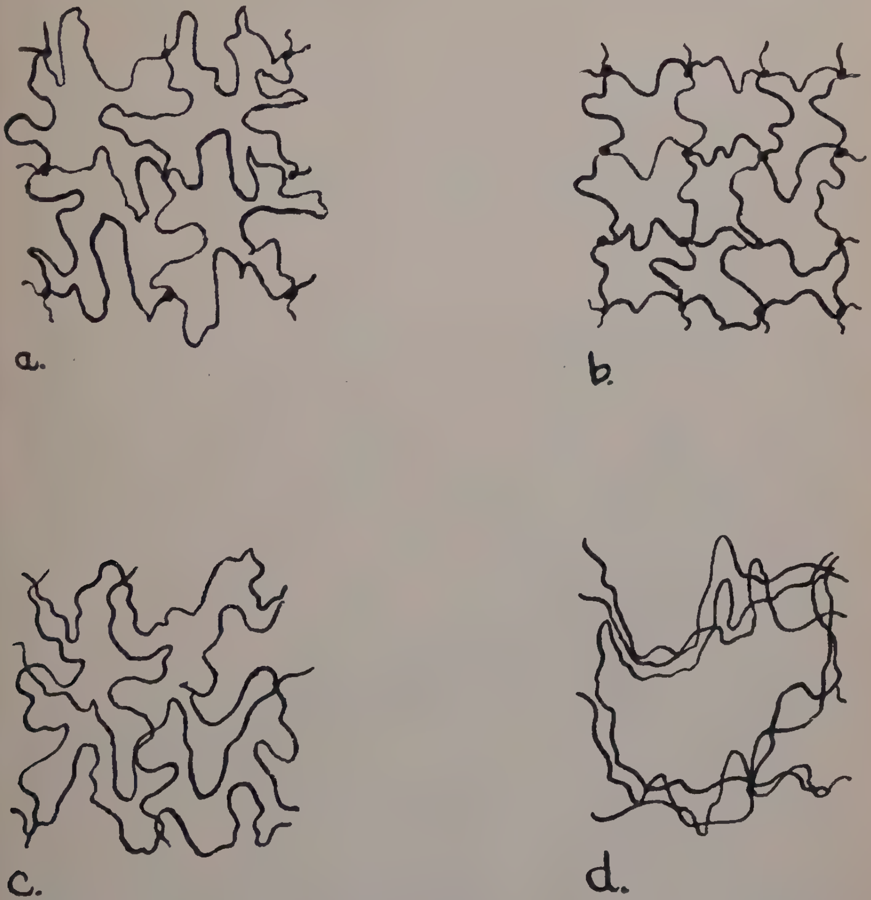

FIGURE 6. Diagrams showing configurational entropies of four polyelectrolyte systems having the same composition: a, average crosslinked; b, high crosslinked; c, "ideal" linear; d, real linear.

$\pi \leqq 0$ for DVB 2, then the sorption curve of this resin can serve as a valid base for the calculation of thermodynamic osmotic pressures.

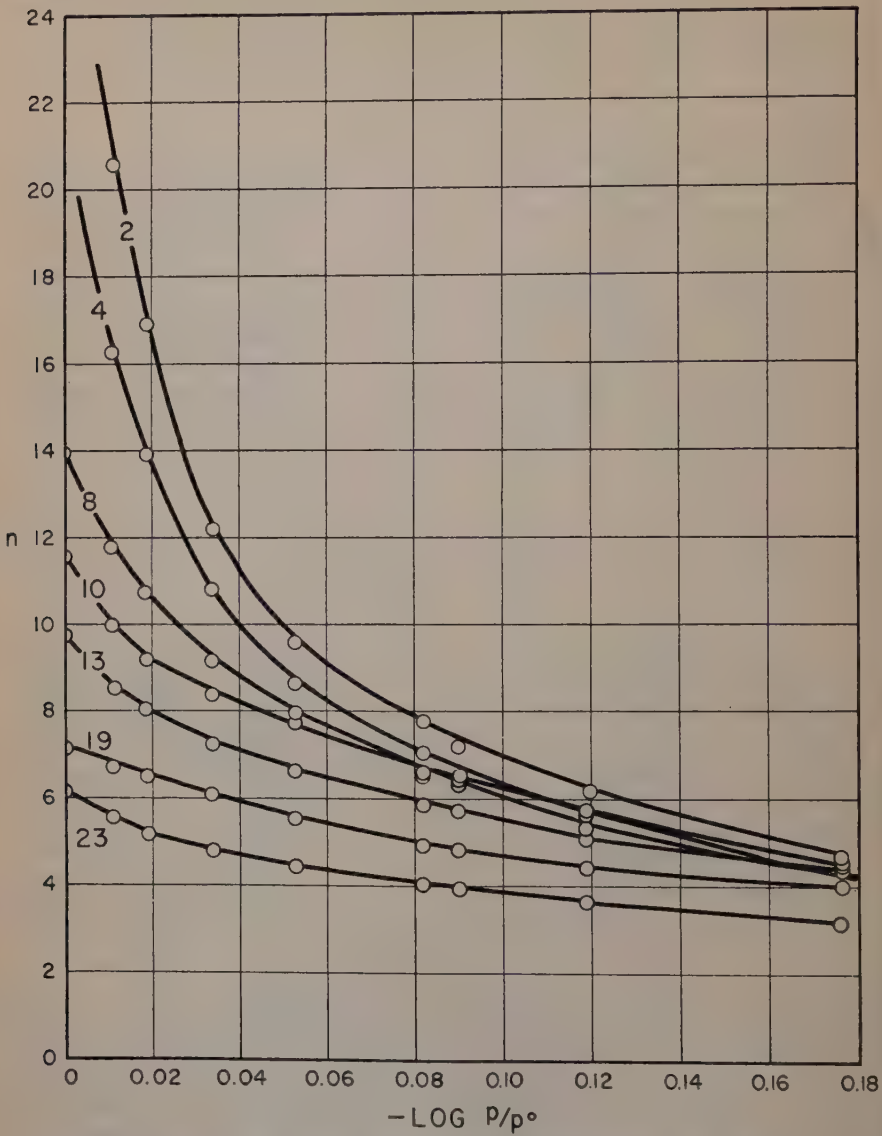

FIGURE 7. Moles of water sorbed, n, plotted against −log p/p° (from data of FIGURE 5).

Calculations of π were performed by first plotting n as a function of $-\log p/p^o$, as shown in FIGURE 7, and reading values of $\pi \bar{V}_w$ from the horizontal displacements. Values of $\bar{V}_w$ were determined pycnometrically.[4] These values vary from 16.8 to 17.6 ml./mole, depending upon the exchange cation present. For sulfuric acid, the value is 17.2 ml./mole. Similar plots and calculations were made for resins in other exchange states, taking in each case the data for DVB 2 in that state as a base line ($\pi \cong 0$). Values of V, the external volume of the resin, were determined pycnometrically.[5]

A plot of π as a function of the degree of crosslinking is shown in FIGURE 8, for resin systems all in equilibrium with dilute solutions. Here it is seen that π increases almost linearly with the per cent crosslinking. Also, π increases with the size of the exchange cation, as expected. A $\pi - V$ plot is shown in FIGURE 9. Here it is seen for a given degree of crosslinking that π is indeed quite linear with V, as postulated previously. Also, lines drawn through systems having the same exchange cation (K^+, Li^+, Me_4N^+, Et_4N^+, Bu_4N^+) describe regular hyperbolas. The striking similarity between these experimentally determined curves and those calculated previously for a simple model, FIGURE 3, does not require comment. A more complete set of $\pi - V$ relationships is shown in FIGURE 10. Again, a straight line relationship between π and V is observed. The position of the hydrogen state resin is anomalous.

The values of the two parameters in FIGURE 3 for the rest volume V_o, and the elastic modulus m were taken from the data of FIGURE 9, so that a meaningful comparison could be made. An *a priori* calculation of V_o and m from independent data has not, as yet, been accomplished.

All of the above curves, FIGURES 8, 9, 10, showed relationships for resins which were in all cases in equilibrium with dilute solutions, *i.e.* fully swollen. Values of π can also be calculated for resin systems at lower relative humidities. While linear $\pi - V$ relationships are obtained at higher values of p/p^o, deviations occur at lower values. This is as expected, since anomalous entropy effects are known to take place under the latter conditions. Also, the concentration of the resin phase increases very sharply as p/p^o decreases. For example, resin DVB 8 is 4 molal at 100 per cent relative humidity, 8 molal at 80 per cent, and 12 molal at 60 per cent. When π is plotted, however, as a function of the square root of the molality of the exchange cation, an excellent straight line relationship is obtained. This is shown in FIGURE 11. No explanation is offered.

Ion Selectivities

The swelled volume of an ion exchange resin in a particular exchange state, as compared to its volume in another exchange state, is a valuable index of the extent to which the resin preferentially absorbs either ion. Ion selectivities are usually expressed as selectivity coefficients,

$$K_2^1 = \left(\frac{n_1}{n_2}\right)_i \left(\frac{n_2}{n_1}\right)_o$$

where the n's refer to moles of cation in the resin and solution phases. The selectivity coefficient, which is often written K_D, is thus a simplified form of the mass action constant for the exchange process. Gregor[2] has pointed out the solvent exchange must also be considered, this corresponding to a free energy of swelling term. A simplified form of the correct expression is

$$RT \ln \left[K_2^1 \left(\frac{\gamma_1}{\gamma_2}\right)_i \right] = \pi(v_2 - v_1)_i \,,$$

where the ratio of cationic activity coefficients in the resin phase is $(\gamma_1/\gamma_2)_i$, and where v_1 and v_2 are the hydrated molar volumes of the exchange cations.[3] When the difference between v_1 and v_2 is small, any deviation of K_D from unity

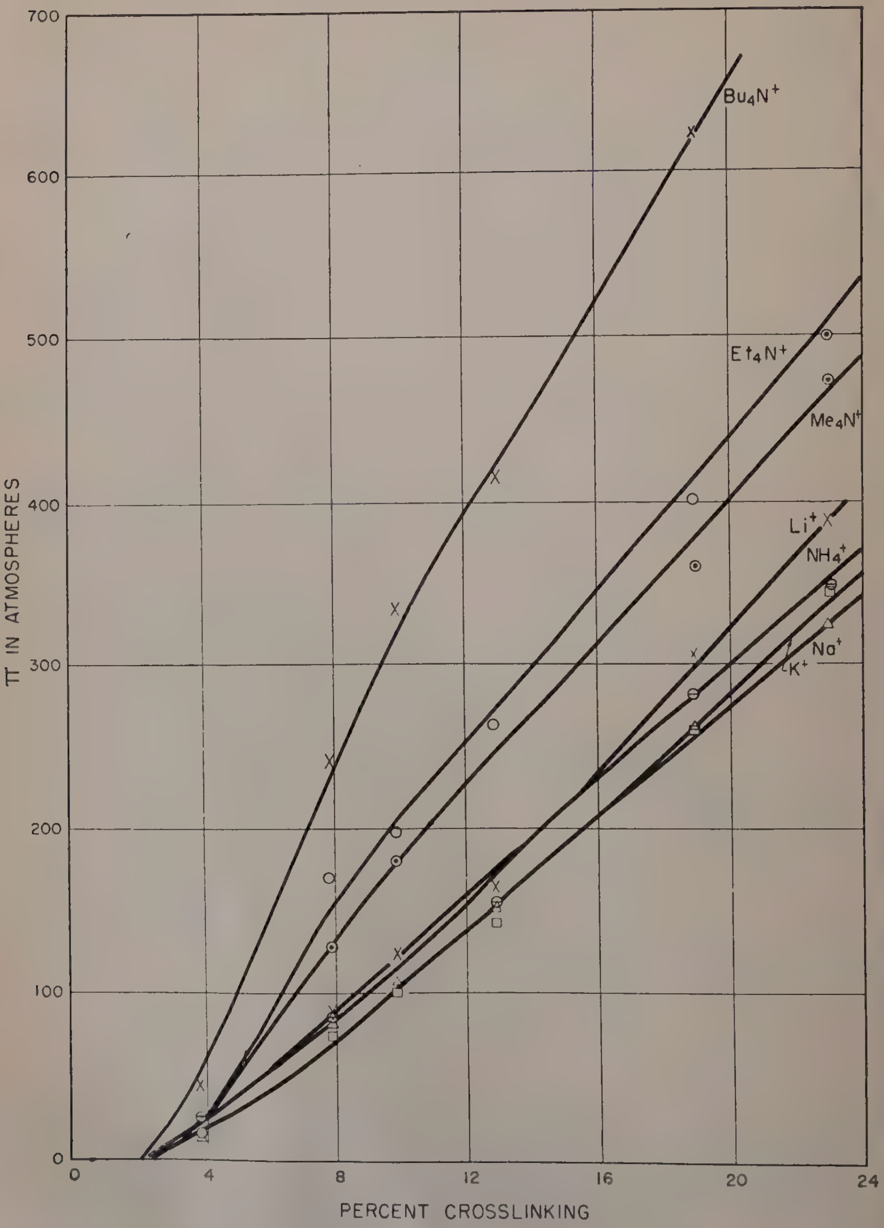

FIGURE 8. Thermodynamic osmotic pressures for polystyrene sulfonic acid resins of varying degrees of crosslinking and in different states.

is ascribed to activity coefficient effects, which will be taken up later. Where the exchange cations are different in size, the pressure-volume correction term may be large.

Selectivity coefficients for various ammonium ions measured against potassium with resins of varying degrees of crosslinking are shown in FIGURE 12. Here it is seen that as the size of the substituted ammonium cation increases,

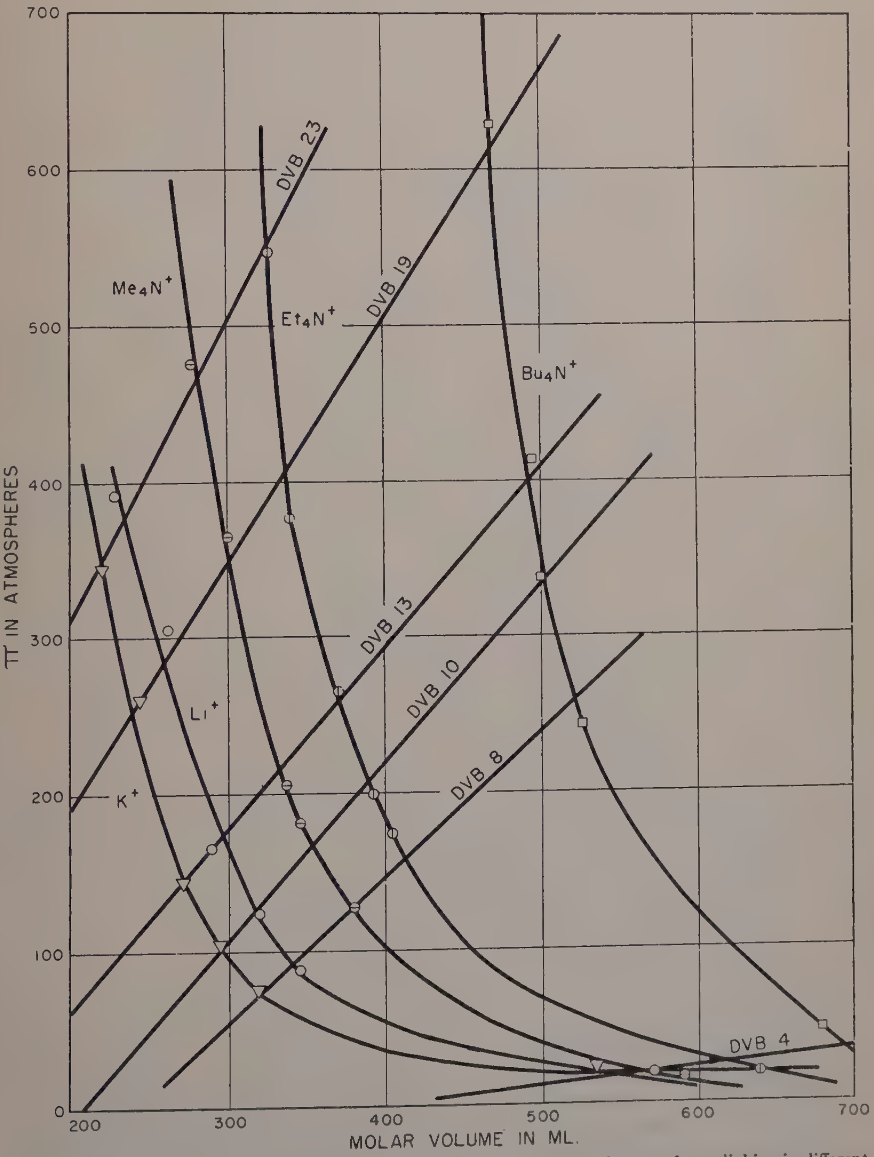

FIGURE 9. Osmotic pressure *vs.* molar resin volume for resins of varying degrees of crosslinking in different exchange states.

the resin prefers the smaller potassium ion more strongly. This effect is not observed with the NH_4^+ ion, because it is approximately the same size as the hydrated K^+ ion.

The fact that at low crosslinkings the value of K_D drops below unity is ascribed to the adsorption of the large organic ions by the organic resin matrix.

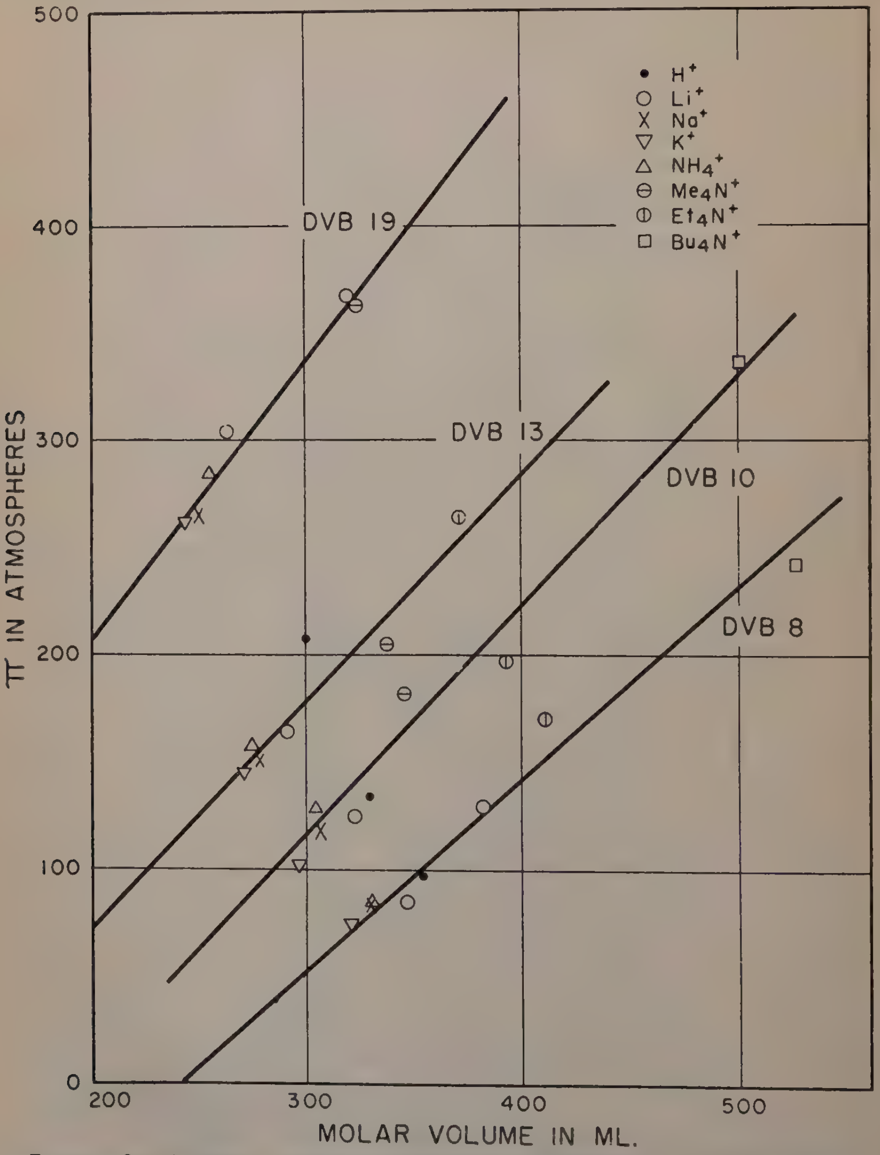

FIGURE 10. Osmotic pressure *vs.* molar resin volume for resins of varying degrees of crosslinking in different exchange states.

This adsorption effect is in the order $Me_3PhN^+ > Bu_4N^+ > Et_4N^+ > Me_4N^+$, as expected. In the exchange isotherm reaction it means that $\gamma_{K^+}/\gamma_{R_4N^+}$ is less than unity, and decreases with the molecular weight of the organic ion.

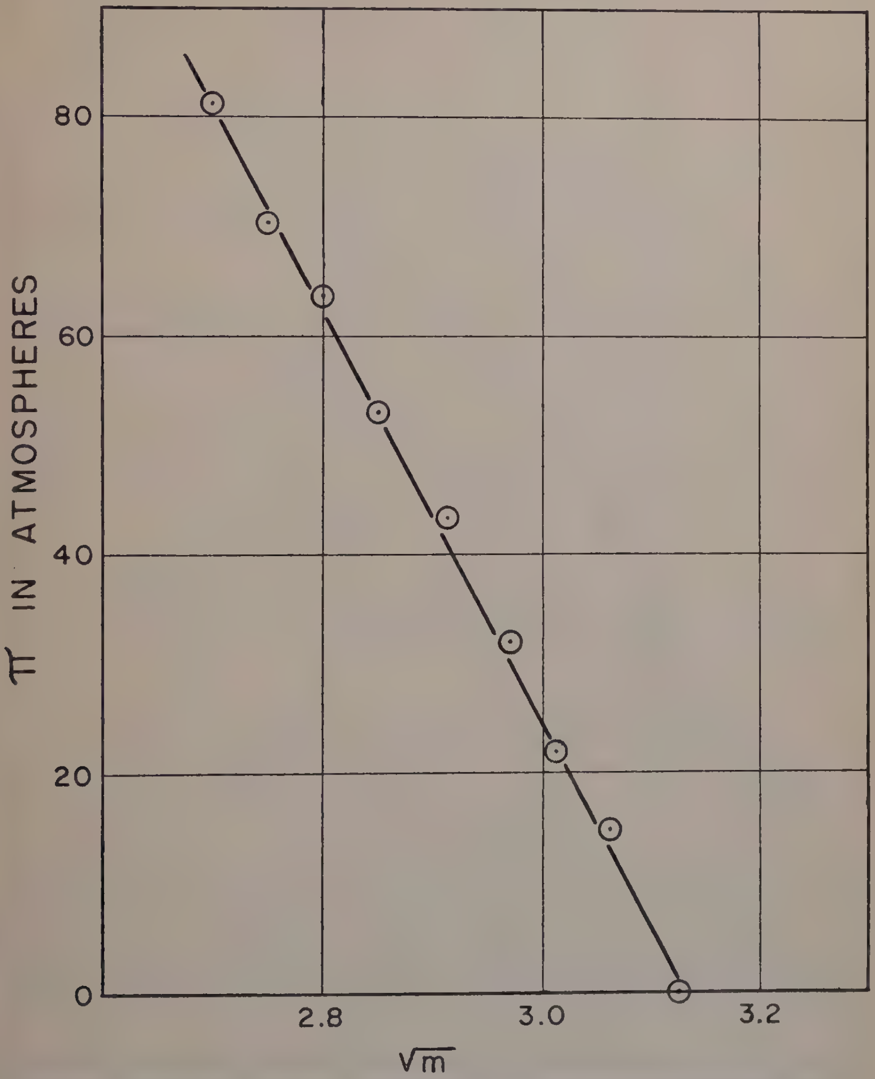

FIGURE 11. Osmotic pressure as a function of the square root of the molality of the exchange ion for resin DVB 10 in the potassium state.

Therefore it is seen from FIGURES 9 and 12 that the osmotic pressure term may be the principal factor in determining ion selectivities. The smaller ion gives the smaller resin volume, corresponding to a lower osmotic pressure.

It is, therefore, preferentially absorbed so that the free energy of swelling is a minimum.

In those cases where the exchange cation can interact with the fixed group, the resin volume also indicates the order of ionic selectivities. This interaction may be the formation of a weakly dissociated ion-pair. FIGURE 13 shows selectivity coefficients for the exchange of alkaline earth ions with potassium. Here the K_D values are plotted against the solubility product of the corresponding sulfate salt. Also, the difference between the swelled volume of the resin in the various alkaline earth metal states and the dry volumes is shown.

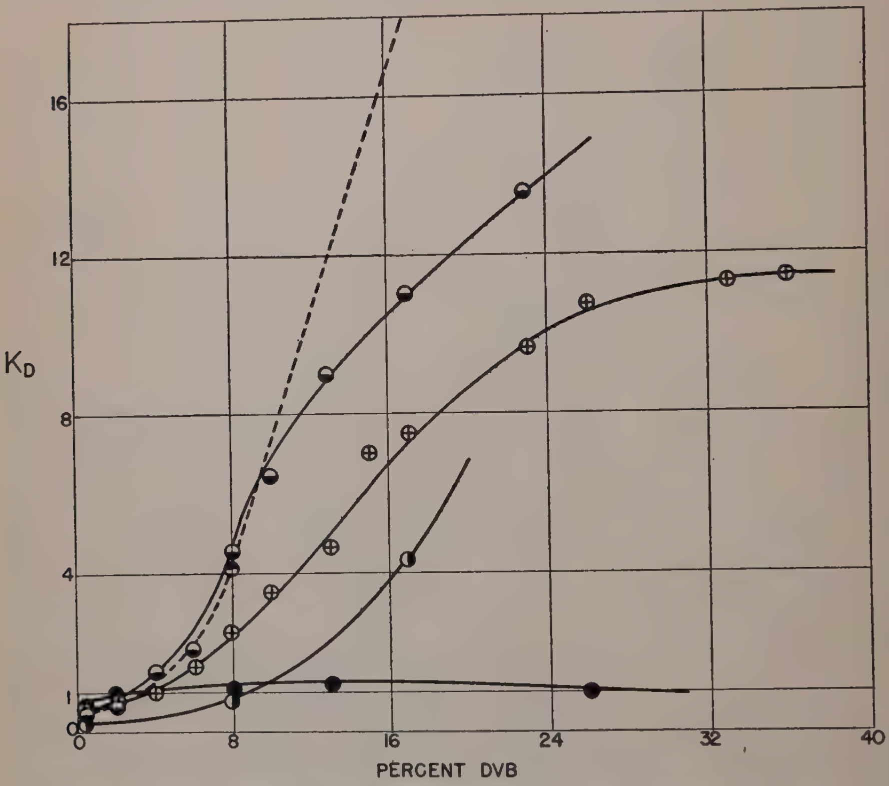

FIGURE 12. Selectivity coefficient measured against potassium with various DVB sulfonic acid resins. Cations are: ●, ammonium; ◑, trimethylphenylammonium; ⊕, tetramethylammonium; ◒, tetraethylammonium; ◓, tetrabutylammonium.

FIGURE 13 shows that the sequence of K_D values is $\mathrm{Ba}^{++} > \mathrm{Sr}^{++} > \mathrm{Ca}^{++} > \mathrm{Mg}^{++}$, which is proportional to the sequence of the log K_{sp} values for the sulfates. This suggests strongly that this is also the sequence of increasing degrees of dissociation of the alkaline earth metal-sulfonate ion-pairs. The sequence of swelled resin volumes $\mathrm{Mg}^{++} > \mathrm{Ca}^{++} > \mathrm{Sr}^{++} > \mathrm{Ba}^{++}$ confirms this. The

Mg form of the resin demonstrates little association or ion-pair formation, the Ba form shows strong ion-pair formation.

In conclusion, it has been demonstrated that the swelling properties of ion exchange resin systems are valuable indices of the exchange processes themselves. Whether exchange ions vary in their properties because of their hydrated sizes, or in their abilities to interact with the fixed exchange groups themselves, these phenomena manifest themselves in the degree of swelling.

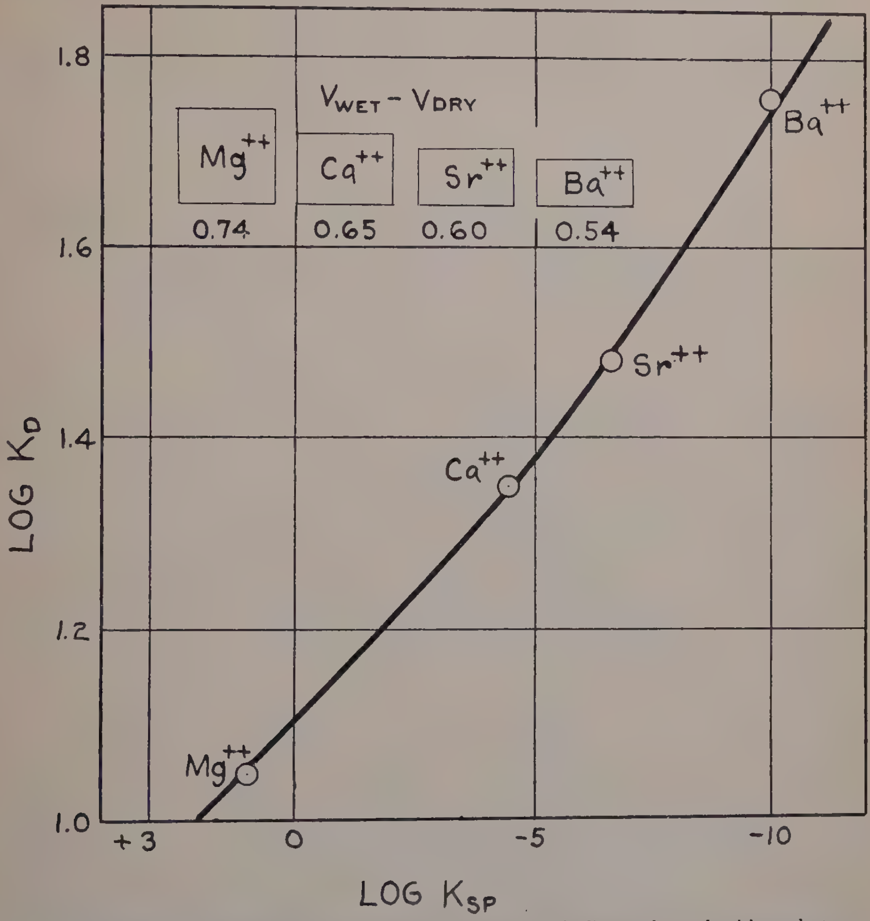

FIGURE 13. Selectivity coefficients for exchange of potassium with alkaline earth metals with a polystyrene sulfonic acid resin (DVB 10). Selectivity coefficients are plotted against the logarithm of the solubility product constant for the alkaline earth metal sulfates. The relationship between K_D and the swelled resin volume is shown.

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EQUILIBRIUM CONSTANTS OF CATION EXCHANGE PROCESSES*

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University of Kansas, Lawrence, Kansas

The program of research on ion exchange in our laboratory has been directed toward the determination of thermodynamic equilibrium constants for cation exchange reactions of the type

$$A^+ + B\text{Res} = B^+ + A\text{Res}$$

where A^+ and B^+ are present, together with a common anion, in aqueous solution, while ARes and BRes are the components of the resin phase.

For a given batch of resin, the value of such an equilibrium constant, which in this simplest case where both cations are univalent may be formulated as

$$K = \frac{a_{B^+}a_{A\,\text{Res}}}{a_{A^+}a_{B\text{Res}}}$$

in which a represents the activity of the designated component, should be independent not only of the equilibrium composition, with respect to the two cations involved, of resin and of solution, but independent also of the total initial concentration of the solution. Furthermore, it should be possible to calculate the equilibrium constant for a given exchange reaction from those of two other exchange reactions. Thus if ARes, BRes, and CRes have all been made from the same batch of exchange material, such as Dowex 50 of a given divinylbenzene content, then in the three exchanges

$$A^+ + B\text{Res} = B^+ + A\text{Res} \tag{1}$$

$$C^+ + B\text{Res} = B^+ + C\text{Res} \tag{2}$$

$$C^+ + A\text{Res} = A^+ + C\text{Res} \tag{3}$$

which for convenience we may designate as a "triangular" system, the equilibrium constants must be logarithmically additive. That is, if K_1, K_2, and K_3 are the respective equilibrium constants for EQUATIONS 1, 2, and 3, the following relationship must be valid:

$$\log K_3 = \log K_2 - \log K_1$$

$$K_3 = K_2/K_1$$

Since both the equilibrium liquid phase and the equilibrium resin, considered as a solid solution, are nonideal solutions, the true equilibrium constant K is related to the equilibrium quotient K_m (the proper quotient of molalities, m, in the liquid solution and resin component mole fractions, N, in the solid phase)

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* The authors wish to express their appreciation of a grant from the Office of Ordnance Research, United States Army, which made possible some of the work described in this paper.

as shown in the following equations:

$$K_m = \frac{m_{B+} N_{A\,\mathrm{Res}}}{m_{A+} N_{B\,\mathrm{Res}}}$$

$$K = K_m \left(\frac{\gamma_{BX}}{\gamma_{AX}}\right)^2 \frac{f_{A\,\mathrm{Res}}}{f_{B\,\mathrm{Res}}}$$

Here γ denotes the mean activity coefficient of an electrolyte in the aqueous solution (X is the common anion in this solution) and f the activity coefficient of a resin component on a mole fraction basis, with pure resin as the standard state.

It will be convenient to define also a third quantity, K_a, which we shall refer to as the apparent equilibrium constant, as the equilibrium quotient corrected for nonideality in the solution but not for nonideality in the solid phase. The relationships among K_m, K_a, and K are shown in the following equations:

$$K_a = K_m \left(\frac{\gamma_{BX}}{\gamma_{AX}}\right)^2 \qquad (4$$

$$K = K_a \frac{f_{A\,\mathrm{Res}}}{f_{B\,\mathrm{Res}}}$$

In the course of our work, several refinements of earlier practice have successively been put into effect. Each of these, we believe, has constituted a step in the direction of the ultimate object stated in the opening sentence of this paper.

The first refinement consisted in the more accurate determination of equilibrium conditions; *i.e.*, of the concentrations of the two cations in the aqueous solution and their relative proportions in the resin at equilibrium. From the results of our earliest series of investigations,[1] it became apparent that the equilibrium composition of the resin phase could not, as had been supposed by numerous investigators, be accurately calculated from the known capacity of the resin and the differences between the initial and the equilibrium concentrations of the cations in the solution. Actually, the total process occurring when a resinous ion exchanger is introduced into a mixed aqueous solution includes several phenomena other than simple exchange of ions.[2] The main reason for the nonequivalence of exchange is the selective withdrawal of water from the solution by the resin. The liquid held within its structure, while not indeed pure water, yet contains ordinarily an appreciably smaller concentration of solute than does the external solution, which therefore undergoes a measurable increase in concentration during the exchange process. In all but this first series of exchange studies in our laboratory, the compositions of both the solution and the resin phase were determined by means of direct analysis for both cation components.

The second refinement to be introduced consisted in the evaluation and application of the activity coefficient ratio factor, $\frac{\gamma_{BX}}{\gamma_{AX}}$. Although activity coefficient values for most of the more common individual salts are available in the literature, data for activity coefficients (or even for activity coefficient ratios, which are all that is required for our present purpose) in mixed solutions are available in a few instances only. As a first approximation, the ionic strength principle of Lewis and Randall[3] may be applied in the following slightly modified and less restrictive form: the *ratio* of the activity coefficients of two given strong electrolytes is the same in all dilute solutions of the same ionic strength. In solutions of 0.1 molal ionic strength, this principle is applicable without appreciable error. Even in 0.2 molal solutions, however, it can no longer be regarded as accurately valid.

Activity coefficients in mixed aqueous solutions have been experimentally determined in a relatively small number of instances, comprising for the most part solutions of alkali halides with the corresponding hydroxides or halide acids.[4] The exchange system which has been most intensively studied in our laboratory is that involving sodium and hydrogen ions in chloride solutions. Fortunately for our purpose, then, activity coefficient data are available for sodium chloride–hydrochloric acid mixtures, which behave in accordance with what has come to be known as Harned's rule:[4] in mixed solutions of constant total ionic strength, the logarithm of the activity coefficient of either electrolyte is a linear function of the concentration of the other. This rule, for 1-1 electrolytes, may be expressed by means of the equations

$$\log \gamma_{AX} = \log \gamma^{o}_{AX} - \alpha_{AB} m_{B+} \tag{5}$$

$$\log \gamma_{BX} = \log \gamma^{o}_{BX} - \alpha_{BA} m_{A+} \tag{6}$$

in which γ_{AX} and γ_{BX} are the mean activity coefficients of the two electrolytes in mixed solutions of total molality m, in which the individual molalities are m_{A+} and m_{B+}, respectively ($m_{A+} + m_{B+} = m$), and γ^{o} denotes the activity coefficient of the electrolyte in question in a pure solution of molality m in that electrolyte. The coefficients α_{AB} and α_{BA}, which are constant at a given temperature for solutions of constant total molality, are known for sodium chloride–hydrochloric acid mixtures of total molality from 0.1 to 3.[4]

Another exchange which has been studied in our laboratory is that involving silver and hydrogen ions. These investigations were made on mixed aqueous solutions of silver nitrate and nitric acid. Since no information on the activity coefficients of the components of such solutions was available in the literature, the necessary data were obtained experimentally.[5] By the measurement of the electromotive forces of appropriate cells containing silver nitrate–nitric acid solutions as electrolyte, the *ratio* of the activity coefficients of the two electrolytes was determined as a function of solution composition for solutions of total ionic strengths from 0.1 to 1.0 molal.

The third refinement in the determination of the true equilibrium constant consisted in the evaluation of the activity coefficients of the resin components. These may be found from the exchange data themselves, when the activity coefficient ratios of the electrolytes in the solution are known, by a method which was described independently and almost simultaneously in a communication from this laboratory[6] and in two papers by Ekedahl, Högfeldt and

Sillén.[7] For the simplest case, involving a pair of univalent ions, this method may be outlined as follows. Since, from EQUATION 4,

$$\ln K = \ln K_a + \ln f_{A\mathrm{Res}} - \ln f_{B\mathrm{Res}} \tag{7}$$

and K is by definition constant, we may write

$$d \ln f_{A\mathrm{Res}} - d\ln f_{B\mathrm{Res}} = -d\ln K_a$$

Now the Gibbs-Duhem equation for the solid phase may be written

$$N_a \, d\ln f_{A\mathrm{Res}} + N_B \, d\ln f_{B\mathrm{Res}} = 0$$

Solution of these two differential equations as simultaneous equations yields the relationships

$$d\ln f_{A\mathrm{Res}} = -N_{B\mathrm{Res}} \, d\ln K_a \text{ and}$$

$$d\ln f_{B\mathrm{Res}} = N_{A\mathrm{Res}} \, d\ln K_a$$

from which, by integration, we obtain the following equations:

$$\ln f_{A\mathrm{Res}} = -(1 - N_{A\mathrm{Res}}) \ln K_a + \int_{N_{A\mathrm{Res}}}^{1} \ln K_a \, dN_{A\mathrm{Res}}$$

$$\ln f_{B\mathrm{Res}} = N_{A\mathrm{Res}} \ln K_a - \int_{0}^{N_{A\mathrm{Res}}} \ln K_a \, dN_{A\mathrm{Res}}$$

Further, the determination of the value of K now presents no further difficulty, since by substitution of these values of the activity coefficients of the resin components in EQUATION 7 we obtain the equation

$$\ln K = \int_0^1 \ln K_a \, dN_{A\mathrm{Res}}$$

The indicated integrations may readily be carried out graphically on plots of log K_a *vs.* $N_{A\mathrm{Res}}$, such as those shown in FIGURE 1, which are based upon data for sodium-hydrogen exchange at 25° on a sample of Dowex 50 of 10.5 per cent divinylbenzene content, in mixed sodium chloride–hydrochloric acid solutions (AX = NaCl, BX = HCl) of 0.1 M, 0.3 M, and 1.0 M total ionic strengths.[8]

Since the activity coefficients of the resin components at a given resin composition should be independent of the concentration of the aqueous solution in equilibrium with the resin, these three curves might be expected to coincide. Such coincidence would, of course, lead to a single value for K. Actually there is a slight divergence among the curves (a possible explanation for which will be discussed later), and integration leads to values of 1.66, 1.68, and 1.73, respectively, for the equilibrium constant. The values of the activity coefficients of the resin components, for round values of the mole fraction of sodium resin at intervals of 0.1 unit, are plotted on a single diagram, shown in FIGURE 2. These results are sufficiently consistent, it is believed, to support the general validity both of our data and of our methods of calculation.

From data for the silver-hydrogen exchange at 25° on a similar sample of

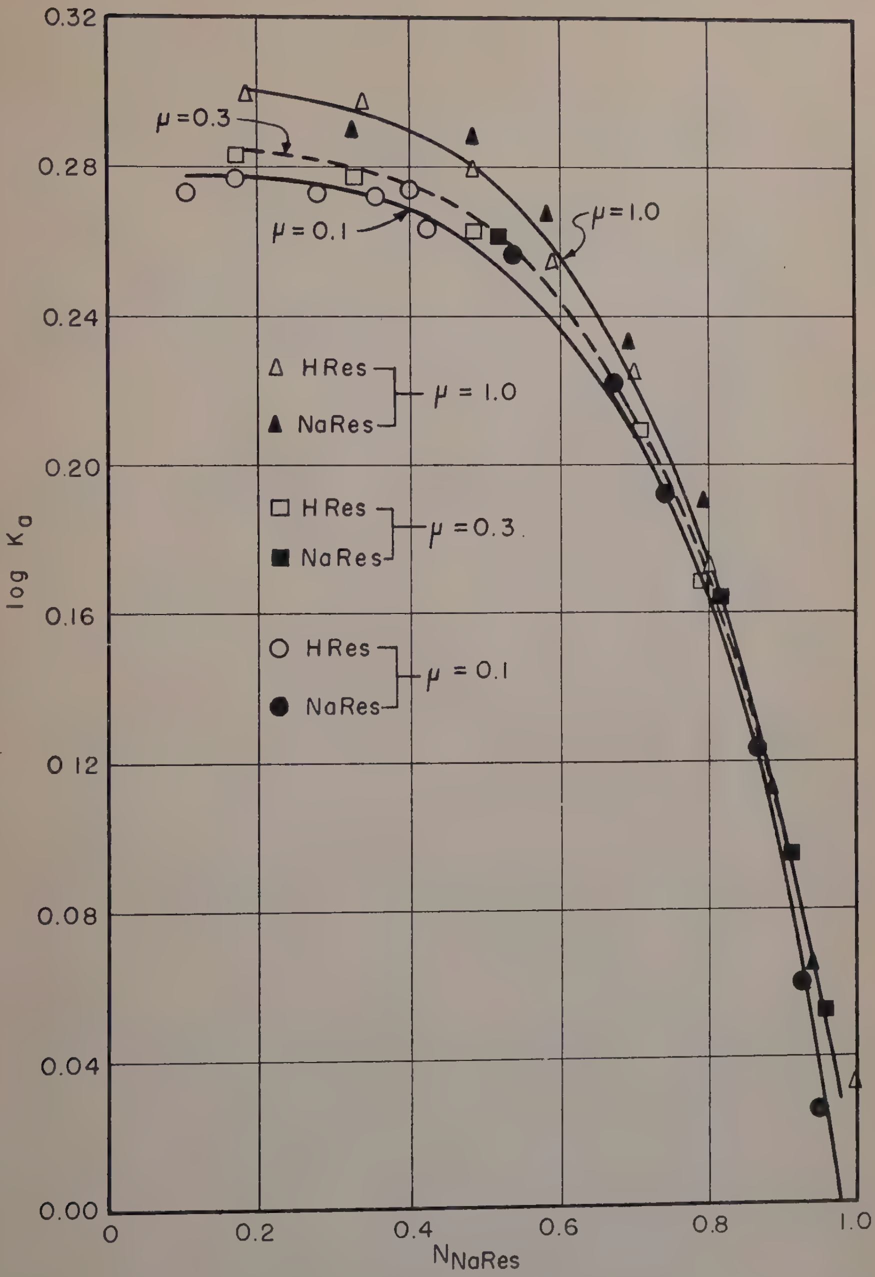

FIGURE 1. Sodium-hydrogen exchange at three ionic strengths.

Dowex 50 in mixed silver nitrate–nitric acid solutions at 0.3 *M* and 1 *M* total concentrations,[8] together with our experimental values for activity coefficient ratios in such solutions,[5] values of K_a were determined, and plots of log K_a *vs.* N_{AgRes} were then used, in the manner just described, to calculate the value of the equilibrium constant for this exchange, see TABLE 1. Exchange data for solutions of 0.1 *M* total concentration are not yet available.

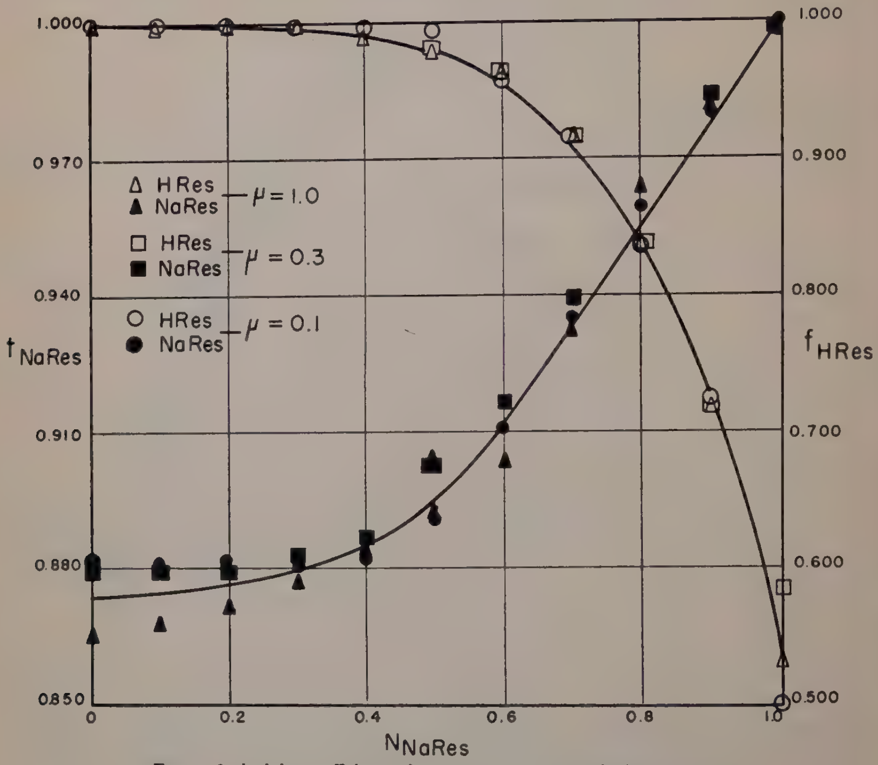

FIGURE 2. Activity coefficients of components of sodium-hydrogen resin.

Data have been obtained also for the sodium-silver exchange on the same resin from silver nitrate–sodium nitrate solutions of 0.1 *M*, 0.3 *M*, and 1.0 *M* total concentrations. In this case, however, activity coefficients are neither available in the literature nor readily accessible to experimental determination. Hence, in order to determine K_a values, it was necessary to have recourse to the Lewis and Randall ionic strength principle;[3] that is, the ratio of the activity coefficients of the two electrolytes in the mixed solutions was taken to be the same as the ratio of their activity coefficients when each is present in its own pure solution at the same total concentration. Since it is unlikely that this principle is still valid for solutions as concentrated as 1 *M*, the K_a values for the exchange in the most concentrated solutions studied are to be regarded as approximate only.

In TABLE 1 are listed values of K for the three exchanges which have been discussed. In each case, the value shown corresponds to the displacement of the second ion from the resin by the first.

Although the agreement among the several values of K for a given exchange is far better than had been attained previously, it is not yet entirely satisfactory, and we now propose a fourth refinement in the method of determining this quantity. Since each form of the resin has its own specific and reproducible capacity for water, an exchange reaction must necessarily include the transfer of a small but finite amount of water between the external solution and the resin phase. Hence, if we represent by n, which we shall call the saturation constant, the number of moles of water taken up per gram-equivalent of resin in its completely wet, saturated, or swollen form, we may formulate the exchange process by the equation

$$A^+ + B\text{Res}\cdot n_B\ \text{H}_2\text{O} = B^+ + A\text{Res}\cdot n_A\ \text{H}_2\text{O} + (n_B - n_A)\ \text{H}_2\text{O} \qquad (8)$$

TABLE 1

EQUILIBRIUM CONSTANTS FOR CATION EXCHANGE REACTIONS

Total molality	K_1 Na–H	K_2 Ag–H	K_3 Ag–Na	
			Observed	Calcd., K_2/K_1
0.1 *M*	1.66	—	7.03	—
0.3 *M*	1.68	11.7	7.52	6.96
1.0 *M*	1.73	13.7	(9.7)	(7.9)

In harmony with this formulation, we may now regard the pure *water-saturated* resin as the standard state, for each variety of the resin. Experiments in our laboratory[8] on water absorption by mixed ammonium-hydrogen and sodium-hydrogen resins showed the water content of a water-saturated resin to be a linear function of the mole fraction of either resin component, accurately in the former case, nearly so in the latter. We shall assume this linear relationship to be at least approximately valid for all equilibrium mixtures of two univalent cation forms of a sample of Dowex 50 of fixed divinylbenzene content. On this assumption, any such mixed resin may be regarded as consisting of *two* components only, which may conveniently be taken to be the two pure water-saturated resins. It is true that the saturation constants n_A and n_B may be expected to vary with the water activity, and hence with the ionic concentration, of the external solution. Experimental work done elsewhere on this problem,[9, 10] as well as a few experiments in this laboratory, however, appear to indicate that such dependence, at least up to molal concentrations, is but slight for a resin of 10 per cent divinylbenzene content. More important for our present purpose is the fact that the difference between the saturation constants of two forms of the resin is scarcely dependent upon water activity. Hence we shall use in the present discussion the saturation constants for hydrogen and sodium resins which have been found in our laboratory by direct measurement of water uptake by the pure resins: $n_{\mathrm{H}} = 8.05$, $n_{\mathrm{Na}} = 6.68$.

For the modified formulation of the exchange process, EQUATION 8, we have as the corresponding expression for the thermodynamic equilibrium constant

$$K = \frac{a_{B^+} a_{A\,\mathrm{Res}}}{a_{A^+} a_{B\,\mathrm{Res}}} a_W^{(n_B - n_A)}$$

in which a_W is the activity of water in the solution. If we now define a new apparent equilibrium constant, $\bar{K}$, by means of the equation

$$\bar{K} = K_m \left(\frac{\gamma_{BX}}{\gamma_{AX}}\right)^2 a_W^{(n_B - n_A)} \tag{9}$$

it follows that

$$K = \bar{K} \frac{f_{A\,\mathrm{Res}}}{f_{B\,\mathrm{Res}}} \tag{10}$$

The expression for K is thus in a form identical with that obtained previously in EQUATION 4, with the new quantity $\bar{K}$ replacing the K_a of the earlier treatment. The combination of EQUATION 10 with the Gibbs-Duhem equation for the resin phase, and the subsequent integration, may now be carried out exactly as in the previous case, leading to the analogous results:

$$ln\, f_{A\,\mathrm{Res}} = -(1 - N_{A\,\mathrm{Res}})\, ln\, \bar{K} + \int_{N_{A\,\mathrm{Res}}}^{1} ln\, \bar{K}\, dN_{A\,\mathrm{Res}}$$

$$n\, f_{B\,\mathrm{Res}} = N_{A\,\mathrm{Res}}\, ln\, \bar{K} - \int_{0}^{N_{A\,\mathrm{Res}}} ln\, \bar{K}\, dN_{A\,\mathrm{Res}}$$

and $ln\, K = \int_0^1 ln\, \bar{K}\, dN_{A\,\mathrm{Res}}$

For the evaluation of $\bar{K}$, γ_{AX} and γ_{BX} in the mixed solutions may be expressed as before, in terms of Harned's rule. It will now be convenient, however, to introduce the composition variable x, defined as m_B/m. In terms of this variable, EQUATIONS 5 and 6 become

$$\log \gamma_{AX} = \log \gamma^{o}_{AX} - \alpha_{AB} m x \tag{11}$$

$$\log \gamma_{BX} = \log \gamma^{o}_{BX} - \alpha_{BA} m (1 - x) \tag{12}$$

The activity of the water may now be evaluated analytically by application of the Gibbs-Duhem equation to the solution, which leads upon integration to the following expression:[4]

$$\log a_W = \log a_{W(AX)} + \frac{m^2}{55.51} [2\alpha_{AB} x - (\alpha_{AB} + \alpha_{BA}) x^2]$$

in which $a_{W(AX)}$ is the activity of water in a solution of AX alone at molality m.

Expressing this activity in terms of the osmotic coefficient, φ, of the AX solution, we may write

$$\log a_W = -\frac{2m\varphi_{AX}}{2.303 \times 55.51} + \frac{m^2}{55.51}\left[2\alpha_{AB}x - (\alpha_{AB} + \alpha_{BA})x^2\right] \qquad (13)$$

Now from EQUATION 9 we have

$$\log \overline{K} = \log K_m + 2(\log \gamma^{\circ}_{BX} - \log \gamma^{\circ}_{AX}) + (n_B - n_A) \log a_W$$

Substitution in this equation of the values of log γ_{AX} and log γ_{BX} from EQUATIONS 11 and 12, and of log a_W from EQUATION 13 gives

TABLE 2

CONSTANTS FOR SODIUM CHLORIDE-HYDROCHLORIC ACID SOLUTIONS

m	γ°_{NaCl}	γ°_{HCl}	α_{NaH}	α_{HNa}	φ_{NaCl}
0.1	0.778	0.796	−0.055	0.043	0.9319
.3	.710	.755	−0.057	.038	.9212
1.0	.656	.809	−0.058	.032	.9378

$$\begin{aligned}\log \overline{K} = \log K_m + 2(\log \gamma^{\circ}_{BX} - \log \gamma^{\circ}_{AX} - \alpha_{BA}m) - \frac{2(n_B - n_A)m\varphi_{AX}}{2.303 \times 55.51} \\ + 2m\left[\alpha_{AB} + \alpha_{BA} + \frac{(n_B - n_A)\alpha_{AB}m}{55.51}\right]x \\ - \frac{(n_B - n_A)(\alpha_{AB} + \alpha_{BA})m^2}{55.51}x^2\end{aligned} \qquad (14)$$

This equation may now be applied to the sodium-hydrogen exchange on a sample of Dowex 50 at three different ionic strengths, with A denoting Na and B denoting H. We shall assume the difference between n_B and n_A to be constant and equal to 1.37, while the other constants, taken from Harned and Owen,[4] have the values listed in TABLE 2.

With these values of the constants, we obtain from EQUATION 14 the following expressions for log $\overline{K}$:

$m = 0.1$

$$\log \overline{K} = \log K_m + 0.00926 - 0.00243\,x + 0.000003\,x^2$$

$m = 0.3$

$$\log \overline{K} = \log K_m + 0.002465 - 0.01165\,x + 0.000042\,x^2$$

$m = 1.0$

$$\log \overline{K} = \log K_m + 0.09799 - 0.05486\,x + 0.000642\,x^2$$

The values of log $\overline{K}$ computed from all of our exchange data are plotted against

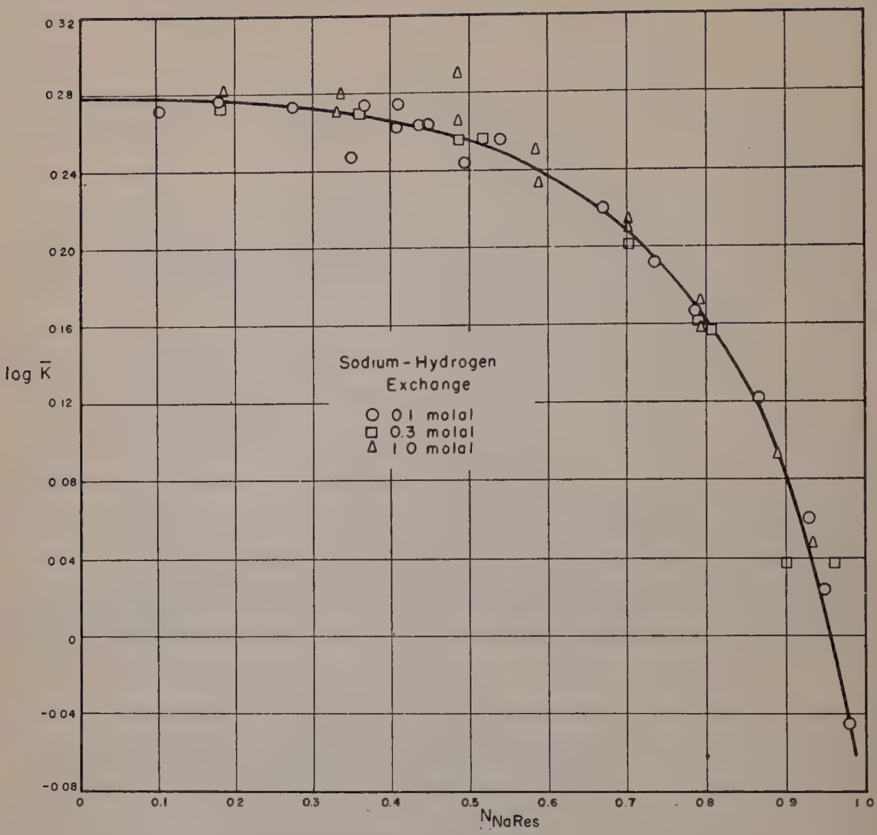

FIGURE 3. Corrected apparent equilibrium constants for sodium-hydrogen exchange.

TABLE 3

ACTIVITY COEFFICIENTS OF *NaRes* AND *HRes* IN MIXED RESINS

N_{NaRes}	f_{NaRes}	f_{HRes}
0.	0.863*	1.000
.1	.869	1.000
.2	.874	0.999
.3	.878	.997
.4	.882	.995
.5	.888	.989
.6	.905	.967
.7	.928	.925
.8	.956	.853
.9	.995	.733
1.0	1.000	.507*

* Extrapolated values.

N_{NaRes} in FIGURE 3. Since, if our hypotheses are correct, we should now expect all of these points, regardless of the ionic strength of the solution, to lie on a single smooth curve, the line in FIGURE 3 has been drawn so as to give approximately equal weight to every experimental point except those two or three which are obviously in error. It may be seen that all of the points fall fairly well on this curve. The values of the activity coefficients of the resin components at round values of N_{NaRes}, as obtained by graphical integration, are listed in TABLE 3.

The value of the true equilibrium constant, K, as found from the curve of FIGURE 3, is 1.64. This value might be expected to agree with the value calculated by the earlier method for infinitely dilute solutions, where the water activity becomes unity and $\overline{K}$ becomes identical with K_a. It is evident that the value found from FIGURE 3 is in accord, within experimental error, with that which would be obtained by extrapolation of the K_1 values of TABLE 1 to zero ionic strength.

It is planned to extend this method to other systems when sufficient data become available.

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THE KINETICS OF ION EXCHANGE PROCESSES*

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Introduction

The subject of ion exchange kinetics has commanded a considerable amount of interest for several reasons. In the first place, the actual time involved in an exchange process is of practical significance when one is faced with a separation problem. Of paramount interest, however, is the fact that kinetics may offer another tool in elucidating the structure and behavior of ion exchange media. Considering the similarity between ion exchange gels and those found in living systems, any information obtained may be useful in investigating biochemical processes.

One of the first serious studies of ion exchange kinetics was conducted by Nachod and Wood,[1] who investigated the hydrogen-calcium system in a relatively dilute range (.01–.001 N). They found that the rate controlling step was a bi-molecular process governed by the law of mass action.[2] Later, Kunin and Myers[3] studied a series of weak base anion exchangers and concluded that the rate-controlling step was the diffusion of the ion through the particle. A considerable amount of light was shed on the factors governing the rate processes of ion exchange by the work of Boyd, Adamson and Myers.[4] They proved that diffusion is the rate controlling step and postulated three types of diffusion mechanism: (1) With solutions 0.1 M or higher in total electrolyte the rate was controlled by particle diffusion; (2) With solutions .003 M or lower the rate was controlled by diffusion through film at the periphery of the particle; (3) In the intermediate range a mixed diffusion process took place. Kressman and Kitchener,[5] using a batch technique in contradistinction to the shallow bed technique used by Boyd, investigated the kinetics of both inorganic and large complex organic molecules in ion exchangers. They found that the energy of activation for the inorganic ion processes was approximately 5000 calories but that, in the case of benzylphenoldimethyl ammonium ion, the value was 8000 calories. It would thus appear from their work that, for the inorganic ions, the differences in rates of diffusion could not be explained by changes in energies of activation. Following this study, Reichenberg[6] investigated the sodium-hydrogen exchange for a series of differently crosslinked exchangers. He found a definite decrease in the rate of diffusion as the resin was increasingly crosslinked, while the energy of activation showed only a slight increase with crosslinking. Reichenberg therefore concluded that the major reason for the change in rate was the change in the steric properties of the exchanger, the energy of activation differences with crosslinking being too small to account for the decrease in the rates. It would appear, therefore, that the rate-controlling step in an ion exchange process is the diffusion of the ion in one of three methods: (1) Film diffusion in which ions are transported

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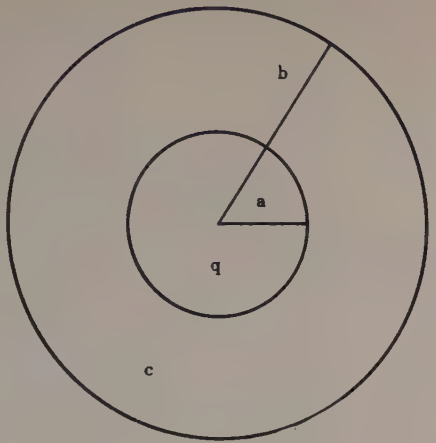
* This work was performed for the Atomic Energy Commission. Some aspects of the work are covered in greater detail in papers that will be submitted to the Journal of the American Chemical Society.

EQUATIONS FOR SELF-DIFFUSION INTO A WELL-STIRRED BATH



Radial Diffusion Equation:

$$\frac{\partial(Rq)}{\partial t} = D^i \frac{\partial^2(Rq)}{\partial R^2}$$

Boundary Conditions:

a) Sphere initially at constant concentration

$q = q_o \qquad R < a \qquad t = o$

b) Solution initially devoid of radioactivity

$c = o \qquad R > a \qquad t = o$

c) Surface of sphere in equilibrium with solution

$q = kc \qquad R = a \qquad t > o$

d) Conservation condition

$$\frac{dc}{dt} = - \frac{4\pi a^2 D^i}{V^l} \left(\frac{\partial q}{\partial R}\right)_{R=a} \qquad t > o$$

Solution of radial diffusion equation:

$$\text{(I)} \quad F = 1 - \frac{2}{3W} \sum_{1}^{\infty} \frac{e^{-\lambda_n^2 \tau}}{1 + \lambda_n^2/9W\,(1 + W)} \qquad \text{for } \tau > 0.1$$

where $\tau = D^i t/a^2 \quad ; \quad W = kV^r/V^l$

$$\text{(II)} \quad F = \frac{(W + 1)}{W} \left\{ 1 - \frac{1}{\alpha - \beta} \left[\alpha e^{\alpha^2 \tau} \, (1 + \operatorname{erf} \alpha\sqrt{\tau}) - \beta e^{\beta^2 \tau} \, (1 + \operatorname{erf} \alpha\sqrt{\tau}) \right] \right\} \text{for } \tau < 0.1$$

where α and β are the roots of $X^2 + 3WX - 3W = 0$

FIGURE 1. Equations for self-diffusion into a well-stirred bath. (Drawing 14020.)

across the boundary separating the exchanger and the outside media; (2) Particle diffusion whereby the rate is determined by the penetration of the ion into the exchanger itself; (3) A mixed diffusion process which contains a combination of both of the two processes previously outlined.

The present paper is concerned with the process of particle diffusion and offers only a summary of some of the major factors affecting this process. All the previously mentioned investigations, save those of Boyd, *et al.*, were concerned with the kinetics of actual diffusion into the particles. For several reasons the investigation of a self-diffusion process was considered desirable. Ordinary diffusion into an ion exchanger, contrary to self-diffusion, involves an exchange process wherein one ionic species in the exchanger is substituted by another and thus necessitates continuous changes in the size of the exchanger and the environment therein. Further, self-diffusion concerns a system which is at equilibrium save only for an isotopic unbalance. Since all these factors should exert great influence on the rate of ion migration inside the exchanger, the use of self-diffusion was expected to introduce considerable simplification into the interpretation of kinetic data.

$$F = 1 - \frac{2}{3w} \sum_{1}^{\infty} \frac{e^{-S^2n\tau}}{1 + \frac{Sn^2}{9w(1 + w)}}$$

where $w = K_d \times \text{ratio} \dfrac{\text{of resin particle volume}}{\text{to volume liquid satisfied by it}}$

$\tau = \dfrac{Dt}{a^2}$ a = radius of resin particle

the poles of Sn are roots of

$$s \cot s = 1 + \frac{s^2}{3w}$$

FIGURE 2. Equations for self-diffusion into a well-stirred bath. (Drawing 17941.)

Experimental Procedure

The experimental procedure was essentially a batch operation in which resins of accurately known particle diameters were placed in a 0.22 N solution containing the same cation as the exchanger. The rate process was usually followed by measuring the emergence of activity from the resin particle. On the other hand, the process could be reversed and the rates determined by the entrance of the activity into the exchanger. A considerable amount of study had previously been devoted to the effects of stirring on the rates observed, and it was found that, at stirring rates above 400 r.p.m. for the system used, there was no significant effect upon the rate of exchange. In most of the experiments conducted, a rate of 1000 r.p.m. was therefore used.

In order to determine the self-diffusion coefficients from experimental data, one must employ the solution of the radial diffusion equation for a sphere. A simplified and useful solution has been published by Patterson[7] and was adopted for this study. The boundary conditions in the solution are given in FIGURES 1 and 2 where F is the fractional attainment of equilibrium, w is a function of the experimental conditions used, τ is a variable which is a function

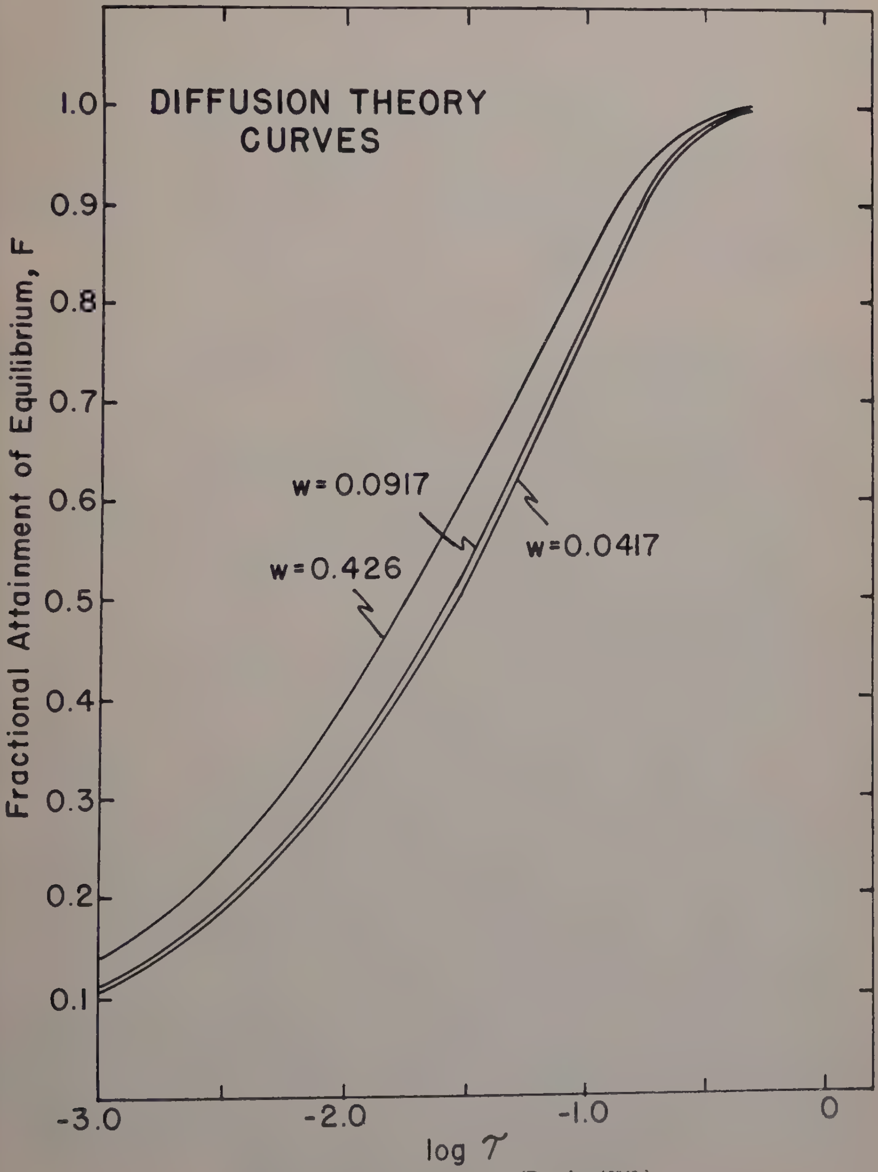


FIGURE 3. Diffusion theory curves. (Drawing 15862.)

of the diffusion coefficient D, time t, and the square of the radius of the particle, a. By inspection one notes that for different values of w a different curve of F against τ will be found. In FIGURE 3 typical curves for values of w from 0.426 to 0.0417 are shown. For the purpose of this study, the conditions were so adjusted that the values of w were approximately .041, although Kressman and Kitchener found that the particle diffusion mechanism still held for values of w approaching unity. In order to calculate a diffusion coefficient one experimentally determines the fractional attainment of equilibrium as a function of time. Then the (w) curve is used to obtain characteristic values of τ for each value of F. By means of photomicrographs an accurate value of the radius of the particle is independently obtained. Therefore insertion of these values in the equation $D = \tau a^2 t$ yields the diffusion coefficient. This procedure was applied to at least fifteen points for each experiment in order to detect any shift in the value of D due to such factors as non-homogeneity of the resin. In most cases the values of D remained constant over a range of F from approximately 0.2 up to a value of 0.9.

Results and Discussion

Having established the procedure which gave reproducible results under varying experimental conditions, a systematic study was made of the structural variables affecting ion exchange rate processes.

Crosslinking and ion charge. FIGURE 4 shows typical curves for the self-diffusion of differently charged species as a function of crosslinking. One notes a marked depression in the observed rates as the resin is more highly crosslinked. At the highest crosslinking the effect is minimized since the curves tend to flatten out. Of primary importance is the effect of charge. Even at low crosslinkings the divalent and trivalent ions do not approach the rates found for monovalent ions at most crosslinkings. It has been found that the rates of diffusion of ions in exchangers fall in characteristic groups of curves dependent upon the charge. There are, to be sure, differences in any charge group but in no case studied do the groups cross over. In TABLE 1 are listed typical self-diffusion coefficients found for the Na^+, Zn^{++} and Y^{+++} at 10 per cent crosslinking. There appears to be roughly a factor of ten separating the rates with each unit change in charge. In order to explain these phenomena recourse is made to the Eyring reaction rate theory where $D = e\lambda^2 kT/h \cdot e^{\Delta S/R} \cdot e^{-\Delta H/RT}$, D being the self-diffusion coefficient, λ the distance of jump of the diffusing ion from one equilibrium position to another, kT/h a frequency factor, and ΔH the energy of activation. Values of the energy of activation obtained by measuring D for at least three temperatures are shown in TABLE 2 for the Zn resinate system.

The question therefore arises as to what factors in the Eyring equation are primarily responsible for the dependence of D upon charge and crosslinking. The values of ΔH is affected by at least two major factors: (1) The work needed to form the hole for the diffusing species as it travels through the exchanger medium; (2) The work needed to move the ion through the high electrostatic force field inside the exchanger. In most ionic systems the work to form a hole in an aqueous medium is reasonably independent of cationic size and therefore is relatively constant for all self-diffusion processes. The second factor is an electrostatic contribution which is affected by the dielectric constant of the medium, the degree of association of the ions and the distance of approach of the ions. From TABLE 2, however, it becomes apparent that although differences in ΔH with crosslinking can explain the change in D for any particular cation system, it cannot explain the tremendous charge effect found on D. The characteristic charge differences, therefore, must be primarily accounted for by changes in $\lambda^2 e^{\Delta S/R}$. It would appear reasonable that the values of λ are strongly affected by the charge; one would expect to find larger values for a monovalent ion than for a trivalent ion. As the crosslinking is increased, moreover, the ions are brought closer together and the values of λ are further reduced. In addition, the value of ΔS is also affected by the degree of crosslinking in the system. As the resin becomes more complex the values of ΔS

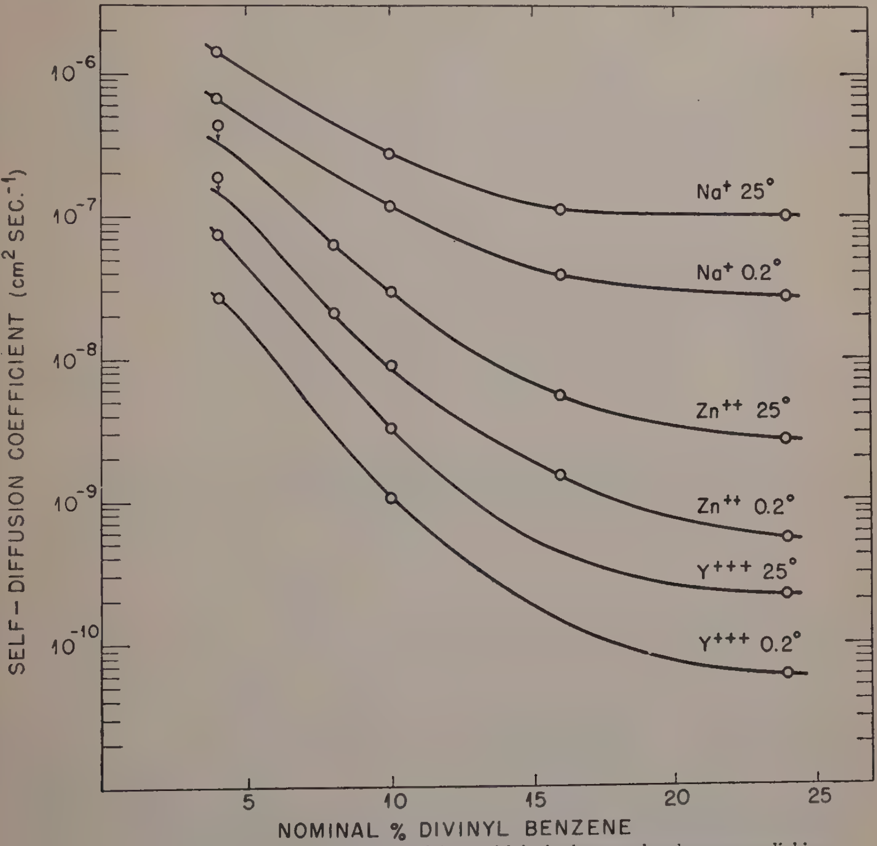


FIGURE 4. Variation of cation self-diffusion coefficients with ionic charge and exchanger crosslinking.

will be reduced. Another factor which reduces the value of ΔS results from the fact that di- and trivalent ions themselves can act as additional crosslinking agents and increase the complexity of the medium.

It should be emphasized that these factors are not the only ones that will effect changes in ΔS and ΔH. Furthermore, because of the extreme complexity in evaluating these parameters no attempt has been made to quantize the magnitudes of each effect. It would appear, however, that several qualitative conclusions can be drawn from the data so far presented: (1) An ion exchanger appears to behave as an aqueous medium since the values of ΔH observed are comparable to those for most inorganic aqueous systems, in contrast to the higher values of ΔH for ion diffusion in solids; (2) The electrostatic force fields inside of the exchanger exert a considerable influence on the movement of ions in the exchanger and constitute the essential difference between usual aqueous systems and highly concentrated ion exchange systems.

TABLE 1

EFFECT OF CHARGE ON THE SELF-DIFFUSION COEFFICIENT

% Crosslinking	Ion	Self-diffusion Coefficient at 25° cm²/sec.
10	Na^+	2.76×10^{-7}
10	Zn^{++}	2.89×10^{-8}
10	Y^{+++}	3.18×10^{-9}

TABLE 2

ENERGY OF ACTIVATION AS A FUNCTION OF CROSSLINKING AND CHARGE

% Crosslinking	ΔH Na^+ Calories	ΔH Zn^{++} Calories	ΔH Y^{+++} Calories
4		5,200	
10		7,700	
16		8,500	
24	8,560	10,000	8,600

Self-diffusion of water. This study so far has dealt with charged species. It was considered to be of some interest to determine the self-diffusion coefficients of uncharged species. Since water plays a fundamental role in all ion exchange processes, it appeared an ideal substance to investigate. A series of sulfonic type resins of varying crosslinking were equilibrated with 0_{18} tagged water, and the rate of emergence of the tagged species into ordinary water was measured for the hydrogen form of the exchangers. At 25° values of 9.14×10^{-6} and 2.2×10^{-6} were obtained for the 4 per cent and 16 per cent DVB systems respectively. As the resin becomes more highly crosslinked the diffusion rates progressively fall off. Further investigation, however, revealed no appreciable effect by crosslinking on the energy of activation for self-diffusion, a reasonably constant value of 5000 calories being observed. The fact that the energy of activation for diffusion of water as a function of crosslinking is constant is in direct contrast with the effect of crosslinking on the ΔH for charged species and further indicates that the changes in energy of activation for charged species as a function of crosslinking is essentially an electrostatic phenomenon.

Capacity. The effect of capacity is also of significance in ion exchange rate studies. Unfortunately, it is difficult to synthesize homogeneous sulfonic type exchangers of varying capacities. To circumvent this difficulty a series of crosslinked sodium forms of ion exchangers was desulfonated at 200°C. By varying the time of exposure in a quartz bomb containing deoxygenated water a series of exchangers of varying capacity can be prepared. This method, however, introduced one serious complication since the question arose as to whether or not crosslinks had been broken. Therefore, a detailed study was made of the water adsorption isotherms of preparations of different capacities and it was noted that below a capacity of four m.eq. per gram dry resin the amount of moisture per m.eq. remained reasonably constant. It was assumed on this basis that in the initial desulfonation process some of the crosslinks were broken but that at a certain point an equilibrium was set up where the scission of the links was balanced by their formation. TABLE 3 indicates the typical behavior of diffusion coefficients and the energy of activation with capacities. Comparing the self-diffusion coefficients of a large number of differently charged ionic species at different capacities indicated one common phenomenon, namely, that with lower capacity, the diffusion coefficients of each system were depressed and the energies of activation for diffusion were increased.

TABLE 3

EFFECTS OF CAPACITY ON THE D AND ΔH OF AG R SYSTEM

H R Capacity	*D.* cm²/sec.		Δ*H* Calories
	0.3°	25°	
4.3	1.23×10^{-7}	3.32×10^{-7}	6,600
3.2	6.84×10^{-8}	1.9×10^{-7}	6,660
2.5	2.24×10^{-8}	6.98×10^{-8}	8,180

TABLE 4

THE EFFECT OF MIXING THE MONOVALENT IONIC RESIN CONTENT ON THE IONIC SELF-DIFFUSION COEFFICIENTS

% of Each Species	M.eq.'s of Each Ion	Ion	*D.* cm² sec.⁻¹		
			0.3°	25°	ΔH
100	3.4 Na R	Na^+	6.58×10^{-8}	2.4×10^{-7}	8,400
1.8	.06 Na R	Na^+	$.97 \times 10^{-7}$	2.62×10^{-7}	6,480
98.2	3.34 Cs R	Cs^+	1.11×10^{-7}	3.1×10^{-7}	6,680
10	.34 Cs R 3.06 Na R	Cs^+	5.42×10^{-8}	1.91×10^{-7}	8,220

Effect of resin composition. Heretofore only systems which contained a single cation have been discussed. As soon as the resin is mixed with another ion, however, a significant effect is noted on the self-diffusion coefficient of the ion. In TABLE 4 are shown the values of D and ΔH in systems containing different ratios of Cs^+ to Na^+. It is seen from this table that the diffusion behavior of sodium in a system predominantly composed of cesium approximates that of cesium. The energy of activation for the diffusion of sodium in such a system is only about 200 calories higher than that found for cesium in comparison with the value of 8400 calories found for the energy of activation of sodium in a system entirely composed of sodium. When the resin environment is completely changed by substituting a trace of cesium in a resin primarily composed of sodium the self-diffusion coefficients of cesium are considerably depressed and the values of ΔH for the cesium process again approximate those found for the pure sodium system. It thus appears that the ionic environment exerts a profound effect on the kinetics of ion exchange processes. This reinforces our conviction that significant rate studies on exchangers must be conducted in a medium where there is no change of ionic environment during the attainment of equilibrium.

In conclusion, it would appear that there are at least four major factors which strongly influence the rates observed in ion exchange systems. They are: (1) the degree of crosslinking; (2) the charge of the ion; (3) the capacity of the resin; (4) the ionic environment of the exchange system (which is itself, undoubtedly, partially affected by the first three factors).

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CATION EXCHANGE PROCESSES

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The advent of cation exchange resins which contain clearly defined functional groups of a single acid type has greatly facilitated the investigation of the nature of cation exchange processes. It is the purpose of this paper to study some of the exchange characteristics of these resins in aqueous solution and, in particular, to correlate and evaluate in a qualitative manner the selectivity data available at present. The discussion will be limited to reactions of univalent cations under equilibrium conditions, since other authors deal with polyvalent cation processes[9] and with the kinetics of exchange reactions.[36]

Cation exchange resins are high molecular weight polyacids which are virtually insoluble in aqueous and most non-aqueous media. They may be pictured as having structures containing large polar exchange groups held together by a three dimensional hydrocarbon network. In support of this picture, it has been calculated[17] that approximately 40 per cent of the hydrated mass of the strong acid exchanger Dowex 50 (Nalcite HCR) is due to the exchange group itself and the water associated with it. The acids which constitute the exchange groups are generally of the sulfonic, phosphonous, phosphonic, carboxylic, or phenolic types. Exchange can be ascribed to the replacement of the dissociable hydrogen ion by other cations.

The number and kinds of exchange groups which are present in a resin may be determined by the "Direct Titration" technique of Gregor and Bregman.[17] This method involves weighing out resin samples in the hydrogen state into a number of test tubes and adding varying amounts of standard base along with a large excess of neutral salt to each. The mixtures are equilibrated with constant shaking, and the pH of the filtered equilibrium solution is then plotted as a function of the amount of base added per gram of oven dry weight of resin. If the direct titration of a sulfonic acid resin in the hydrogen state were attempted in the absence of neutral salt, there would be no pH change in the external solution until the end point of the titration had been reached. The reaction can be written

$$R\text{SO}_3\text{H} + \text{NaOH} = R\text{SO}_3\text{Na} + \text{H}_2\text{O}$$

where R represents the resin matrix. Here the actual neutralization is taking place within the pores of the resin, and the pH changes cannot be measured directly. The addition of a large excess of neutral sodium salt will drive most of the hydrogen ions into the solution phase, and the titration with base will then proceed as if the resin were soluble.

FIGURE 1 shows titration curves for typical polyfunctional resins.[17] In this figure, and throughout this paper, the term meq/gram refers to the oven dry weight of the resin. The resins shown were prepared in the following manner:

(A) By the alkaline condensation of salicylic acid-phenol formaldehyde;[39]

(B) By sulfonating phenol with an excess of fuming sulfuric acid, adding phenol and a slight excess of formaldehyde, and condensing in the acid state;

(C) Phenol-formaldehyde, alkaline condensed, was cured and dried, then treated with sodium sulfite.[38]

The titration curves show that resin "*A*" contains carboxylic and phenolic groups, "*B*" has sulfonic, carboxylic, and phenolic groups, while resin "*C*" has sulfonic and phenolic groups. The sharing of the total capacity by more than one acidic functional group was also illustrated by Boyd, Schubert, and Adamson[5] who, working with a phenol-formaldehyde sulfonic acid resin, found that the sulfonic group accounted for only 75 per cent of the total capacity of the resin. Any attempt to explain the cation exchange processes of resins like these is thus complicated by the presence of two or more types of acid groups.

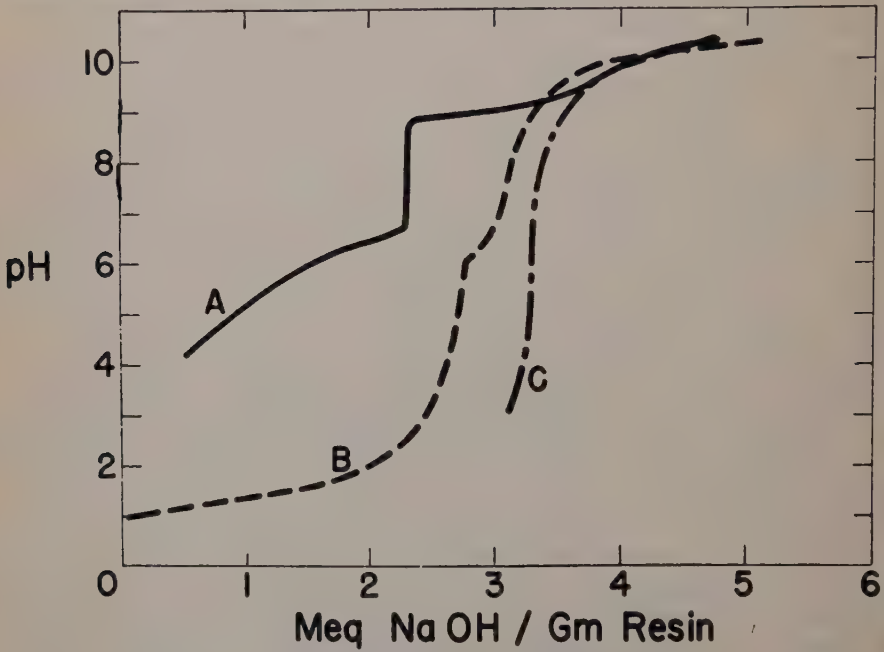

FIGURE 1. Titration curves of polyfunctional cation exchange resins.[17]

In 1947, Bauman and Eichorn[2] described the properties of the monofunctional high capacity sulfonic acid cation exchange resin Dowex 50 (Nalcite HCR), a sulfonated polystyrene-divinylbenzene copolymer. Since that time other high capacity resins which contain only phosphonic or carboxylic groups have been synthesized. In FIGURE 2, titration curves for these three resin types are shown along with one for alkaline condensed phenolformaldehyde[1] which is a monofunctional phenolic type resin. The curve for the carboxylic resin is taken from Kunin and Barry.[27] It can be seen that the pK values indicated for the resin acid groups are similar to those which would be expected for the corresponding monomers. The sulfonic acid resin can be considered as a strong acid resin with its full capacity available for ion exchange purposes at pH values of 2 or higher. The carboxylic and phenolic resins, on the other hand, are weak acid exchangers and require high pH values for their efficient utilization. The phosphonic resin titration curve shows the existence of two replaceable hydrogen ions, the first one comparable with a moderately strong acid, while the second hydrogen is available at high pH values only, thus functioning as if a weak acid were present.

A comparison of titration curves for phosphonous and phosphonic resins is shown in FIGURE 3. It can be seen that the phosphonous resin curve is almost identical with the first half of the titration curve of the phosphonic resin.

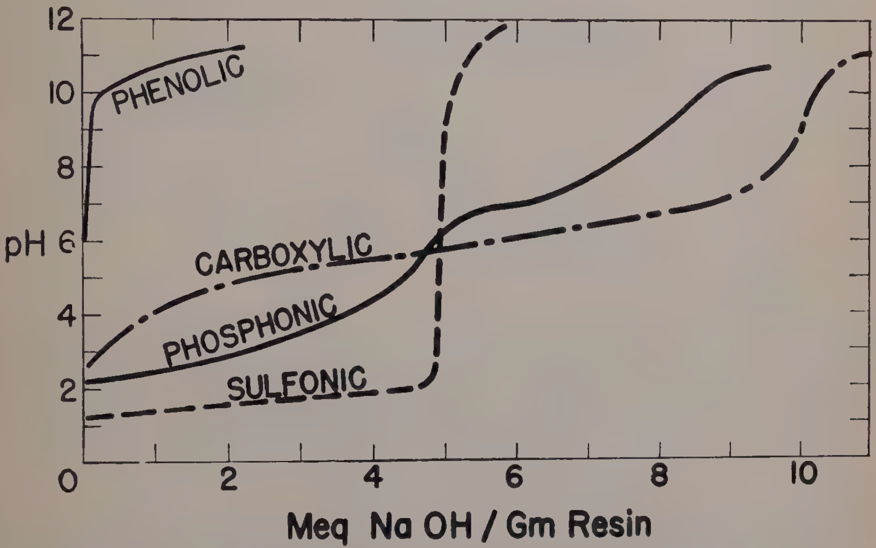

FIGURE 2. Titration curves of representative resin types.

Capacity measurements show that the theoretical capacity of the phosphonous resin for sodium is almost exactly half of the phosphonic value. The phosphonic resin may be written as $RPO(OH)_2$ in a manner similar to the nomenclature recommended by the Committee on Nomenclature, Spelling, and Pronunciation of the American Chemical Society, September 16, 1952. This indicates that two hydrogen ions are available for exchange purposes, a fact borne out by the shape of the resin titration curve. The curve for the phosphonous resin, however, shows only one break, indicating the presence of only one exchangeable hydrogen cation, a fact further shown by the resin capacity. For these reasons, the empirical resin formula should be written as $RPHO(OH)$ or perhaps RPO_2H_2 rather than as the recommended $RP(OH)_2$.

TABLE 1 compares the theoretical capacities for sodium of representative types of cation exchange resins. The phosphonous resin, PM-52, is an experimental batch of Nalco resin. These capacities were obtained by shaking a weighed amount of the resin hydrogen form with a known excess of standard base until equilibrium was reached, and then determining the amount of base neutralized by the hydrogen ions which were released from the resin. These theoretical capacities should be considered as valid only for highly alkaline (pH > 12) solutions. Their values at any other pH's may be approximated from the titration curves. Thus for example, at a pH of 3, the sulfonic resin has the highest capacity, while at a pH of about 5.5 the sulfonic, phosphonic, and carboxylic resins all have equal values, and above this pH value, the latter two have higher capacities than does the sulfonic resin. It should be emphasized here that these are equilibrium capacities. The resin reaction rates are of the order: sulfonic > phosphonic > carboxylic, and this to a considerable degree influences the capacity values obtained under non-equilibrium conditions, *e.g.* column operation.

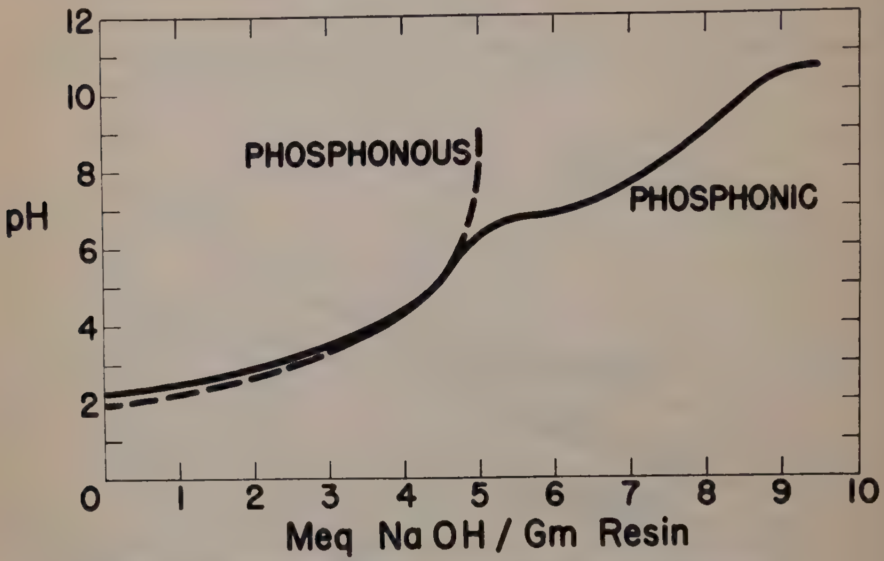

FIGURE 3. Titration curves of phosphonous and phosphonic cation exchange resins.

TABLE 1
THEORETICAL CAPACITIES OF REPRESENTATIVE CATION EXCHANGE RESINS

Resin	Acid Group	Capacity meq./gm.
Nalcite HCR	sulfonic	4.9
Amberlite IRC-50	carboxylic	10.0
Nalcite X-219	phosphonic	8.8
PM-52	phosphonous	4.5

The process of univalent cation exchange may be written as a metathetical reaction:

$$R - X + Y^{+} = R - Y + X^{+}$$

where R represents the resin matrix plus the fixed negative change, and X and

Y are the exchanging cations. This reaction is characterized by two important properties:

(A) Equivalent amounts of cations are exchanged;

(B) The resin generally shows selective uptake for one cation over another.

The exchange of equivalent amounts of cations has been shown by Gregor *et al.*[19] who found the capacity of Dowex 50 to be identical for lithium, sodium, potassium, ammonium, and hydrogen. When resins containing different combinations of sodium and potassium were analyzed for these cations, the total capacity was found to remain constant. Exchange capacities were also found to be identical to a number of univalent organic cations. These tests were all carried out in the presence of a dilute solution phase ($<0.1M$) and were designed so as to avoid the formation of complex ions.

The fact that univalent cation capacity is the same for all ionic species in the case of the sulfonic resin shows that all exchange sites are available, and that molecular adsorption plays a negligible role. This is further evidence in support of the theory that ion exchange resins may be considered to behave like concentrated electrolytic solutions.

For the reaction as written above, one may define the selectivity coefficient as

$$K_y^x = \left(\frac{m_x}{n_y}\right)_i \left(\frac{a_y}{a_x}\right)_o \tag{1}$$

where the n's refer to moles of cations within the resin phase (i), and the a's to the activity of the cations in the external solutions, (o). The notation K_y^x means that ion x is the reference cation.[30] This formulation thus is limited to quantities that can actually be measured experimentally, and differs from the mass-action constant K_a, which is defined as

$$Ka = \left(\frac{a_x}{a_y}\right)_i \left(\frac{a_y}{a_x}\right)_o \tag{2}$$

in that the activities within the resin phase which, as yet, cannot be measured satisfactorily are replaced by the number of moles of cations present. The relationship between these two terms is thus

$$Ka = K_y^x \left(\frac{\gamma_x}{\gamma_y}\right)_i \tag{3}$$

where the γ's refer to the activity coefficients within the resin phase. When K_y^x is used without regard to a particular system, it is written as K_D.[18] The cationic activities are customarily replaced by mean activities, and, in dilute solutions by concentrations, thus allowing the experimental calculation of K_D values.

The experimental techniques involved in carrying out selectivity determinations have been described by many authors[2, 16, 18, 30] and need not be considered in detail in this paper. Unless otherwise specified, all data given here will refer to dilute solutions ($\approx 0.01M$), room temperature, and equivalent mole fractions in the resin phase of the two competing cations. Much of the earlier data on selectivities must be considered to be inaccurate since only the change in the amounts of the cations in the solution phase was measured experimentally. Bonner, Argesinger and Davidson[4] have shown that errors in computation of equilibrium compositions may arise from neglect of the transfer of water between the solution and resin phases during the exchange reaction. In addition, in concentrated solutions ($1M$ or greater) the amount of electrolyte in the resin phase due to Donnan effects becomes considerable and must be taken into account.

Some of the factors which may influence the equilibrium selectivity of cation exchange resins in aqueous solution for univalent cations are the following:

1. Structure of the exchanger;
2. Nature of the acidic functional group;
3. Mole fractions of the exchanging cations in the resin phase;
4. Resin capacity;
5. Ionic strength of the equilibrating solution;
6. Temperature.

The remainder of this paper will be devoted to an analysis of these effects, and a discussion of some of their theoretical implications.

Structure of the Exchanger

Gregor and Bregman[18] have studied the effect of crosslinking on the selectivity characteristics of sulfonated polystyrene-divinylbenzene copolymers. FIGURE 4 shows how the K_D values for ammonium, sodium, hydrogen, and lithium measured against potassium as a reference ion, change with the divinylbenzene content of the resin. It can be seen that at 8 per cent DVB, a composition comparable to that of the commercial resin Nalcite HCR, the order of resin selectivity is $K^+ > NH_4^+ > Na^+ > H^+ > Li^+$. As the degree of crosslinking decreases, the selectivity values begin to approach unity. In the case of the ammonium-potassium system, the resin actually shows greater affinity for ammonium at DVB values of 2 per cent or lower. When the divinylbenzene content is increased, on the other hand, the selectivity for potassium over the other cations increases until it reaches a peak value and then, in the case of the very highly crosslinked resins, drops off sharply. In the lithium-potassium system this drop is so great that at 26 per cent DVB content, the selectivity reverses itself and the resin shows a greater affinity for lithium than it does for potassium.

For a qualitative discussion of selectivity factors, the thermodynamic expression of Gregor[14, 15] may be condensed to the following equation:

$$RT\ ln \left[K_y^x \left(\frac{\gamma x}{\gamma y} \right)_i \right] = \Pi(V_y - Y_x) \tag{4}$$

where R is the universal gas constant, T the temperature, K_y^x has been defined by EQUATION 1, $\left(\frac{\gamma x}{\gamma y}\right)_i$ is the ratio of the activity coefficients of cations x and y in the resin phase $v_y - v_x$ the difference in hydrated molar volumes, and π the osmotic pressure.

In the case of the resins of very low degrees of crosslinking, the pressure-volume term in the equation will be quite small and the K_D values will be determined almost exclusively by the activity coefficient ratio.[18] In the absence of these pressure-volume effects, Ka in EQUATION 3 becomes equal to unity as required by Donnan considerations. If, in the case of the sulfonic resins, γi for each cation is set equal to $\gamma\pm$ for the corresponding monomer, the toluene-sulfonate salt, then it can be seen that $K_D = \dfrac{\gamma_2\pm}{\gamma_1\pm}$. Robinson and Stokes[33] have determined activity coefficients for the alkali salts of toluenesulfonic acid at concentrations up to 4 molal. By utilizing the molalities which have been calculated by Gregor, Gutoff, and Bregman[20] to exist in the sulfonated polystyrene-divinylbenzene copolymers at various degrees of crosslinking, one may compare the ratio of the activity coefficients with the experimentally determined K_D values, as is done in TABLE 2. It can be seen from this table

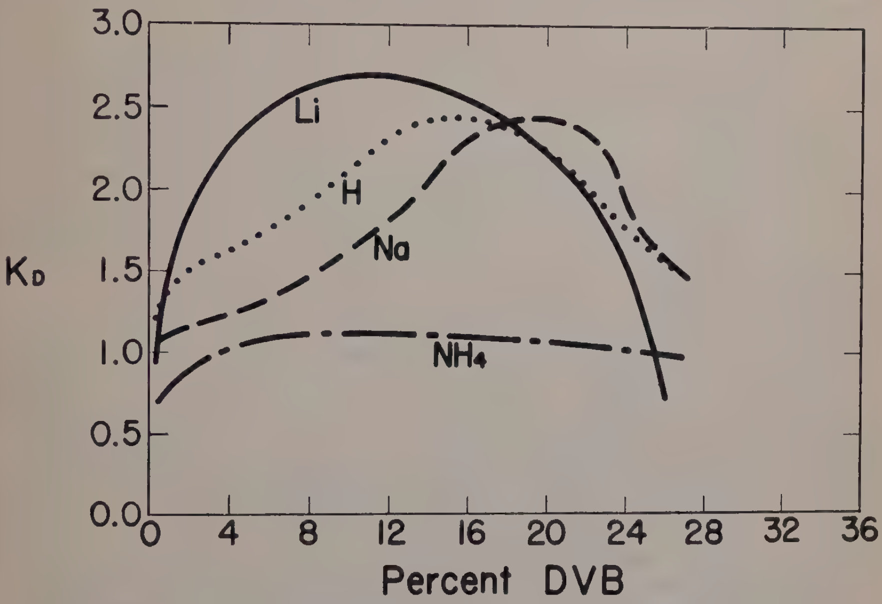

FIGURE 4. Selectivity coefficients of various inorganic cations relative to potassium.[18]

TABLE 2

A COMPARISON OF ACTIVITY COEFFICIENT RATIOS AND K_D VALUES FOR SULFONATED POLYSTYRENE-DIVINYLBENZENE COPOLYMERS OF LOW DEGREES OF CROSSLINKING

Resin DVB Content	Approximate Molality[20]	$\gamma\pm$ Li Tol/ $\gamma\pm$ K Tol[33]	K^{K}_{Li} [18]	$\gamma\pm$ Na Tol/ $\gamma\pm$ K Tol[33]	K^{K}_{Na} [18]
%					
0.4	0.2	1.03	0.96	1.00	1.03
2	1.4	1.33	1.38	1.13	1.16
4	2.4	1.58	2.10	1.25	1.20
6	3.7	1.92	2.08	1.31	1.40

that, for low degrees of crosslinking, the ratio of the activity coefficients agrees surprisingly well with the experimentally determined K_D values. Only one set of results do not agree, those for the potassium-lithium system at 4 per cent DVB, and here the trend of the K_D values seems to indicate that the value of 2.10 is probably in error. It is unfortunate that activity coefficients for the toluene-sulfonate salts are not available at higher concentrations.

As the divinylbenzene content becomes greater (8 to 20 per cent) the pressure-volume terms begin to play an important part in determining the K_D values. The K_D values here rise much more rapidly than would be expected from the little that is known of activity coefficients in these highly concentrated solutions, and the swelling pressures generated by these resins have been demonstrated.[8, 16, 26] Gregor and Bregman[18] have shown that, in this DVB range, Log K_D is proportional to the ratio of the specific external resin volume of the cation being considered to that for potassium. This specific external resin volume had previously been shown to be directly proportional to the hydrated ionic volume of the cation under consideration.[20]

In considering the reversal of selectivity which takes place in the higher DVB resins, *e.g.* for the lithium-potassium system, it should be remembered that the internal molality of the resin phase is of the order of 10 molal. Consequently, as is the case in ordinary concentrated electrolytic solutions, the hydrated volumes of the cations decrease due to a lack of sufficient water to complete the hydration shell. Since the lithium cation has a smaller unhydrated ionic volume and a larger amount of water attached to it than does the potassium cation, it is to be expected that the effective hydrated size of the lithium cation will decrease more rapidly than that of the potassium cation, with a concomitant lowering in K_D. FIGURE 4 showed that the reversal in the direction of K_D appears at lower DVB values for the pairs of ions that have the greatest disparity in their hydrated volumes, potassium and lithium, than it does for the potassium-sodium system where the difference in the amount of hydration is not as great.

FIGURE 5 shows the change in K_D as a function of the DVB content for various organic cations measured against potassium.[18] This graph has been restricted to DVB values of lower than 10 per cent in order to emphasize the effects in this region. If the curves had been continued, it would have been seen that they rise very sharply and do not show any reversal at high DVB values. The organic cations may be considered as practically unhydrated and, at high degrees of crosslinking, the increasing rigidity of the resin matrix prevents it from swelling sufficiently to accommodate many of the large quaternary cations. This is a straightforward example of the effect of the pressure-volume term on K_D.

FIGURE 5 shows that, at DVB contents below 5 per cent, the affinity for the organic cations increases with increasing molecular weight in a manner analogous to the application of Traube's rule to adsorption phenomena. The trimethylphenyl ammonium ion shows particularly strong adsorptive effects because of the similarity of its aromatic character to that of the resin matrix. The preference for this ion over potassium extends all the way to DVB-9, in contrast to the other cations shown in this graph. Since the resin capacity has been shown to be exactly equal to the number of fixed anionic groups,[19] this adsorptive effect cannot be one of molecular adsorption of the salts of the organic ion, or of hydrolytic adsorption, but may be considered to be due to the strong attraction of hydrocarbons for each other. Thermodynamically it may be expressed as a lowering of the activity coefficient of the adsorbed ion. Kressman and Kitchner[25] have shown similar effects with a phenolsulfonic resin and have ascribed the preference for the larger organic cations as being due to van der Waals's forces. Since these forces are only effective at short distances, one would expect them to be strongest in the case of the organic ion which has the most atoms in contact with the resin matrix. TABLE 3, taken from their data, relates the resin affinity to the number of atoms in contact with the surface. Their definition of the equilibrium quotient is the inverse of the definition of K_D as used in this paper and, consequently, in this table the highest values of the equilibrium quotient indicate the greatest resin affinity for the organic cation.

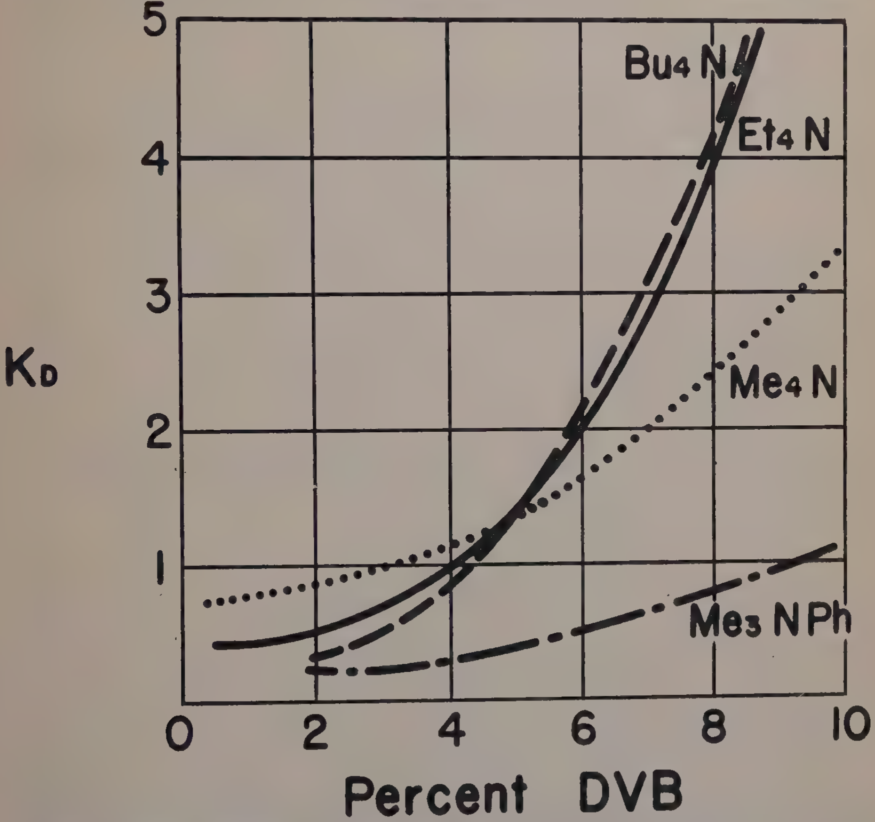

FIGURE 5. Selectivity coefficients of various organic cations against potassium:[18] tetramethyl ammonium; tetraethyl ammonium; tetrabutyl ammonium; trimethylphenyl ammonium.

Gregor and Bernstein[3, 16] have studied the effect of crosslinking on the selectivity characteristics of a series of methacrylic acid–divinylbenzene copolymers. Comparing the selectivity coefficient of the potassium-lithium system with the ratio of the square of the activity coefficients of acetate solutions of similar molalities, they found excellent agreement for the 15.4 per cent DVB resin. The 24 per cent DVB resin showed a qualitative agreement for this comparison. With the 0.99 and 1.94 per cent DVB resins, however, the agreement was poor, and somewhat closer results were obtained when using the ratio of the mean activity coefficients rather than the square of this ratio.

TABLE 3

AFFINITY OF ORGANIC CATIONS FOR A PHENOLSULFONATE CATION EXCHANGE RESIN[25]

Ion	Equilibrium Quotient	No. atoms in contact with Surface*	Calculated Major Diameter
			A°
Ammonium	1.00	0	2.4
Tetramethylammonium	3.67	3	4.6
Tetraethylammonium	5.0	6	7.2
Trimethyl-n-amylammonium	8.24	7	9.5
Phenyldimethylethylammonium	25.2	9	8.5
Phenylbenzyldimethylammonium	44.4	14	11.2

* Not counting hydrogen.

They postulated that the lower DVB resins might be somewhat stronger acids than acetic acid, thus possibly invalidating the use of the acetate activity coefficients. By applying the Duhem equation

$$\left(\frac{\partial \ln a_1}{\partial \ln x_1}\right)_{T,P} = \left(\frac{\partial \ln a_2}{\partial \ln x_2}\right)_{T,P} \tag{5}$$

to this system, they arrived at a final expression

$$\log K_D = \log K_a - k(1 - 2X_K)_i \tag{6}$$

From EQUATION 6 they were able to determine values for K_a. When K_D was then compared with $K_a\left(\frac{\gamma \pm \mathrm{Li}A_C}{\gamma \pm XA_C}\right)$ excellent agreement was obtained.

These authors also studied the selectivity of these resins for potassium against the organic cations tetramethylammonium and tetraethylammonium. They found that the data could be readily explained in terms of pressure-volume effects. Unlike the sulfonated polystyrene-divinylbenzene resins, there was no strong affinity for the organic cations at low degrees of crosslinking. Instead, the selectivity for potassium reached a maximum at about 16 per cent DVB and then decreased. This was explained by the fact that the methacrylic low DVB resins have very little ring structure and hence the effect of adsorption is negligible. As the DVB content increases, however, the ratio of ring structure to polar groups also increases and the resins become increasingly better hydrocarbon adsorbents. At the point of maximum K_D, the pressure-volume effect is apparently counterbalanced by adsorption, and a further increase in DVB content results in a decrease in K_D.

Nature of the Acidic Functional Group

The selectivity of a cation exchange resin for alkali cations is affected by the nature and acidity of the exchange group. TABLE 4 shows how K_D values differ for different types of acidic groups.

The values for the phosphonic and carboxylic resins, Nalcite X-219 and Amberlite IRC-50, were obtained in alkaline solution (pH 12–12.5). Those for the sulfonic resin, Dowex 50 (Nalcite HCR), were obtained in neutral salt solution[2] (pH 7), but checks in alkaline solution have failed to reveal any significant difference.

It can be seen from this table that the weaker acid resins show inverse selectivities to those of the sulfonic resin. This effect is particularly noticeable in the case of the phosphonic resin which shows a strong preference for sodium over potassium, the first cation exchanger to do so. An explanation which has been offered for this reversal of selectivities[7] is based on the work of Teunissen and Bungenberg de Jong[37] dealing with the concentration of alkali chlorides necessary to bring about a reversal of charge on organic colloids. They found that in the case of sulfate colloids the order was, as expected, LiCl > NaCl > KCl, but that in the case of the phosphate colloids this order was inverted. This effect was explained in the following manner:

TABLE 4

SELECTIVITIES FOR ALKALI CATIONS

Cation	K_D for Different Resin Types		
	Sulfonic[2]	Carboxylic	Phosphonic
Li	1.97	0.72	0.65
Na	1.00	1.00	1.00
K	0.80	1.14	1.51

The reversal of charge is generally assumed to be caused by fixation of a sufficient number of cations on the ionized groups. The affinity of cations and the ionized colloidal groups depends, among other things, on the valency and polarizing power of the cation and on the polarizability of the negatively charged ionic group and that of the solvent, water. Polarizability is directly proportional to molecular refraction and, from the data of Fajans,[13] molecular refractions may be calculated which show the order of polarizability to be $PO_4^{\equiv} > COO^- > H_2O > SO_4^=$.

The "field strength" or polarizing ability of the unhydrated alkali cations decreases with increasing ionic size. If the negatively ionized group is more polarizable than water, then the polarization energy and the coulombic energy are additive, and the order of affinity for the negative group is $Li^+ > Na^+ > K^+$. This leads to the observed order of reversal of charge concentrations for phosphate colloids, LiCl < NaCl < KCl. When the polarizability of the ionic group is less than that of water, as with the sulfate colloids, then the lithium cation becomes the most heavily hydrated one, and the order of affinity and that of the reversal of charge concentrations are the inverse of those found for phosphate colloids.

The concept of association in colloids has also been brought out by Wiklander.[40] He concludes that the charge density of the colloid ion as well as the valence and electrodynamic radius of the counter-ions influence the association. Pauli and Valko[29] have assumed that, on association, a partial dehydration of both the colloid ion and the counter-ion takes place at the point of contact. This may lead to polarization of both ions and an increased association as well as to an increase in the replacing power. Consequently, the hydration counteracts the association while the polarizing power of the counter-ion and the polarizability of the colloid-ion favor it.

A phosphonic acid cation exchange resin should have an order of selectivity similar to the order of affinity for phosphate colloids, $Li^+ > Na^+ > K^+$. The tightly-bound three dimensional resin structure should serve to enhance this order of selectivity as the resin should have its smallest volume in the lithium state due to the interaction with the fixed negative group. TABLE 5 shows that the volumes of the phosphonic and sulfonic resins in the alkali states are of the order predicted by selectivity considerations, and that, taking interaction between the cation and the fixed anionic group into account, their relationship is further evidence for the validity of the pressure-volume theory.[15]

TABLE 5
RELATIVE RESIN VOLUME RELATIONSHIP

Resin State	Volume	
	Phosphonic[6]	Sulfonic[20]
Li	96	106
Na	100	100
K	105	96

The resin selectivity may be further compared to the solubility of salts possessing different cations, but the same anion. The solubility sequence of the phosphates[23, 35] in terms of gram formula weights per 1000 grams of water is Li < Na < K, and it would appear logical that the order of decreasing solubility should correspond to the order of increasing resin affinity. Similarly, the order of solubility of the sulfates is K < Na < Li and this agrees with the order of resin affinity for the sulfonated resin, Nalcite HCR.

An approach to the problem of association in weak acid resins[3, 16] which shows the interdependence of polarization, acid strength, and activity coefficients, consists in applying the hypothesis of localized hydrolysis as set forth by Robinson, Stokes, and Harned.[31, 32] By plotting the activity coefficient as a function of the molality for the salts of the alkali metals, they found that the order of the resulting curves for salts of weak acids such as formates, acetates and hydroxides (these being considered as salts of the very weak acid, water) is the converse of that found for the chlorides, bromides, nitrates, *etc.* To explain this reversal of order they postulated that the intense fields around a cation of small radius created an induced polarity of the water in the immediate vicinity. In the absence of any proton acceptor, no further effect would be noted. Thus, in the case of lithium chloride, although the lithium ion is small enough to induce high polarization in a water molecule, nevertheless the chloride ion is a very weak proton acceptor and the activity coefficient curve is "normal." The situation is different with lithium acetate, where the acetate ion is a moderately good proton acceptor and we may imagine a water molecule forming a weak link between the two ions:

$$Li^{+}—HO^{-}—H^{+}—Ac^{-}$$

thereby reducing the total ionic strength and decreasing the activity coefficient calculated in the usual way on the assumption of complete dissociation. The effect should depend upon two factors: (a) The size of the cation, the effect being more pronounced as the cation decreases in size. This is in agreement with the order of the activity coefficient curves: Li < Na < K < Rb < Cs; (b) The strength of the acid from which the salt is derived, the effect becoming larger as the acid becomes weaker. Thus, although γ_{NaX} is greater than γ_{K_x} for salts of strong acids such as hydrochloric, hydrobromic, and hydroiodic acids, the activity coefficient curves of sodium formate and acetate are slightly below those of the corresponding potassium salts, and sodium hydroxide has an activity coefficient curve markedly lower than that of potassium hydroxide.

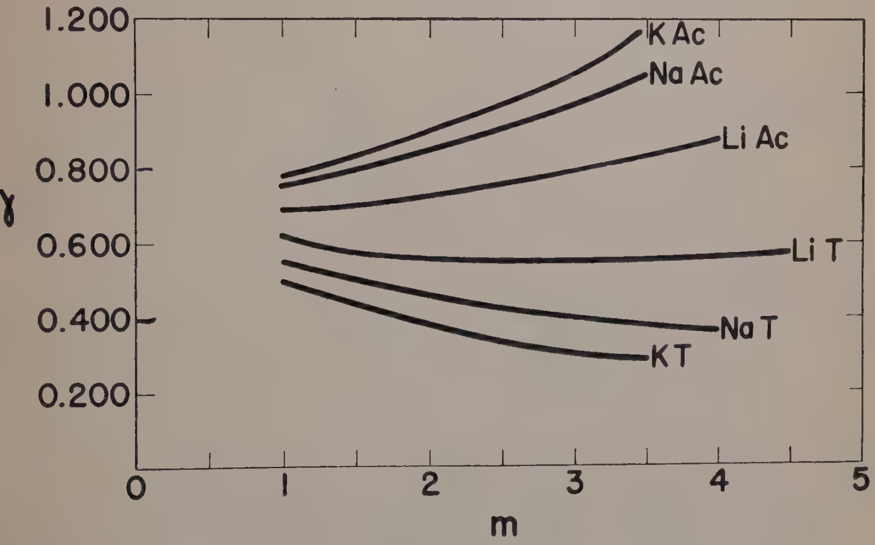

FIGURE 6. Activity coefficients of alkali salts of acetic and toluenesulfonic acids.[33]

FIGURE 6 shows the activity coefficient curves of the alkali salts of acetic and toluenesulfonic acids. It can be seen that the order $\gamma_{Li} > \gamma_{Na} > \gamma_{K}$ for the strong acid is reversed in the case of the weak acid. From the equation $K^{K}_{Li} = \left(\frac{\gamma\pm_{Li}}{\gamma\pm_{K}}\right)_{i}$, one may therefore predict that K^{K}_{Li} for the sulfonic resin will be greater than unity and that for the carboxylic resin less than one. This prediction is borne out by experimental facts.

The phosphonic acid resin titration curve shows that the first dissociation corresponds to that of a moderately strong acid, while the second is that of a very weak acid. If one could isolate the selectivity characteristics due to each dissociation, it would be expected that the first dissociation would lead to selectivities comparable to that of the strong acid resin, Nalcite HCR, potassium, for example, being taken up preferentially over sodium, while the second dissociation would show the selectivity characteristics of a very weak acid group, sodium being taken up very strongly over potassium. In order to test this hypothesis, selectivity studies were carried out at various pH values. FIGURE 7 shows K_D as a function of the pH, while in FIGURE 8 it is plotted against the per cent of the total theoretical capacity utilized at that pH as determined from the titration curve of the resin.

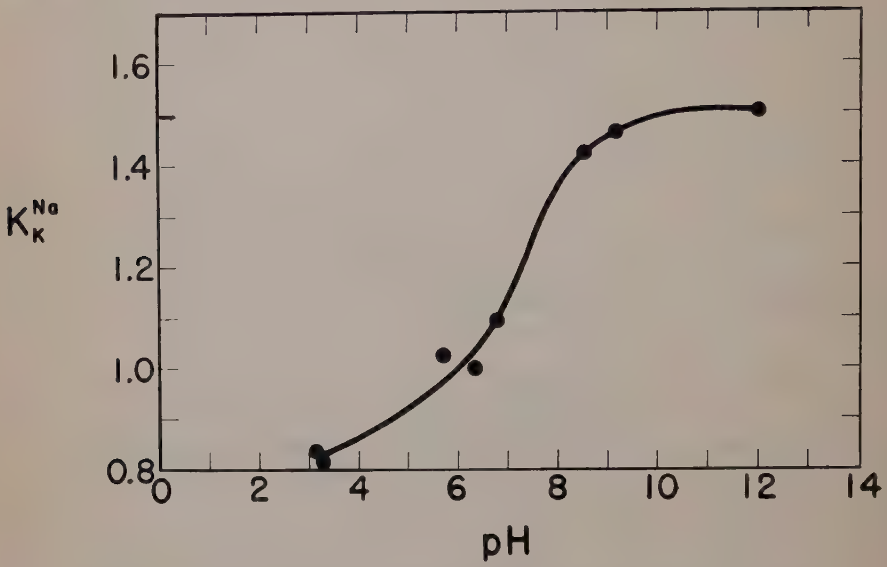

FIGURE 7. Selectivity of Nalcite X-219 for the sodium-potassium system as a function of pH.

TABLE 6

VARIATION OF $\gamma H/_{\gamma Na}$ IN MIXED CHLORIDE SOLUTIONS[30]

Molality	0.1	0.2	1.0	3.0	5.0
$\gamma H/_{\gamma Na}$ $X_{Na} = 0$	1.020	1.033	1.161	1.524	1.951
$\gamma H/_{\gamma Na}$ $X_{Na} = 1$	1.026	1.050	1.308	2.213	3.715

The value of 0.82 for K_D at about 30 per cent of total capacity may be safely taken as that due to the first dissociation alone. Assuming that the value of 1.51 at 100 per cent total capacity is the average of the K_D values due to each dissociation (*i.e.* that the total capacity is divided equally between the two dissociations, an assumption which the titration curves of the phosphonous and phosphonic resin show to be valid) then the K_D value for the second dissociation alone is equal to 2 (1.51) − 0.82 or 2.20 indicating a high selectivity for sodium over potassium, and explaining the increase in the value of K_D as the pH increases and the effect of the second dissociation begins to be of importance.

The discussion of selectivity as a function of the nature of the fixed acid group as been limited to the alkali cations. With the strong acid resin, Dowex 50, hydrogen has been shown to occupy a place in the replacement series which is close to that of lithium.[18] In the case of the weak acid exchangers, however, the selectivity for hydrogen will be a direct function of the degree of resin dissociation, which in turn is a function of the external pH. At any given pH, the weakest acid groups are the ones which are the least dissociated. These resins consequently have a low hydrogen ion activity in the resin phase. This means that a correspondingly lower H-ion activity in the outside solution is required for the maintenance of equilibrium conditions. The replacing power of the hydrogen ion therefore increases with decreasing acidity of the functional group.

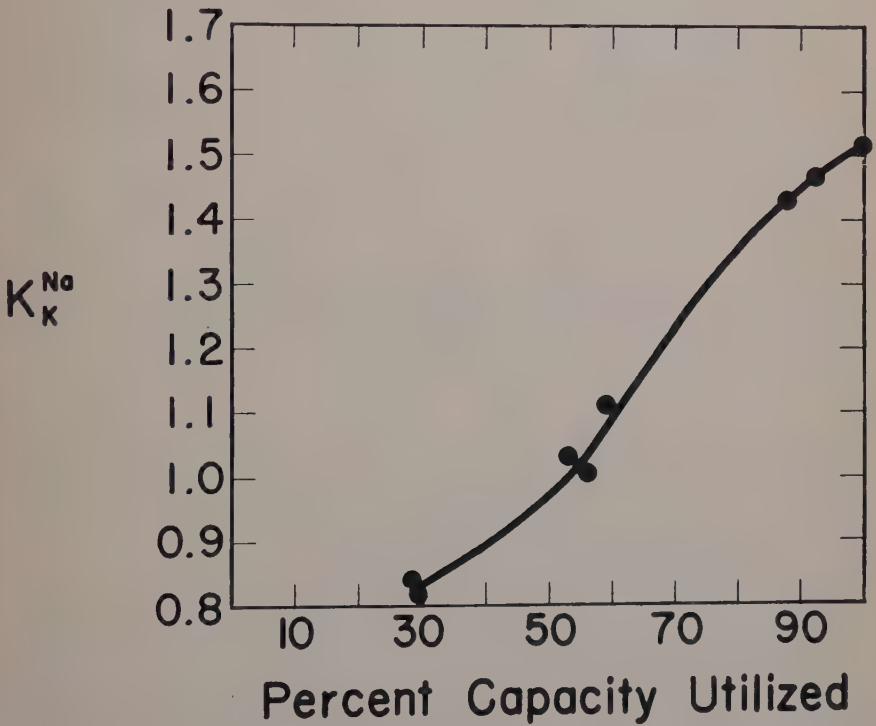

FIGURE 8. Selectivity of Nalcite X-219 for the sodium potassium system as a function of the available capacity.

This discussion has not taken into account the case of univalent cations such as Ag^+, Tl^+, Rb^+, and Cs^+ for which the sulfonic acid resin shows unusually high affinities. The interaction between the resin and these cations probably results in considerable ion-pair formation in the resin with a consequent lowering of the cationic activity in the resin phase.

Selectivity as a Function of Internal Mole Fraction

The variation of K_D with X_i, the mole fraction of one of the cations in the resin phase, has been the subject of considerable study by many investigators.[12, 18, 22, 28, 34] In general, for resins of a conventional degree of crosslinking and a pair of cations which differ significantly in size, it has been found that the affinity for a cation increases as its mole fraction in the resin phase decreases. Typical K_D *vs.* X_i curves are shown in FIGURE 9. Here all K_D values are calculated using potassium as a reference ion. Curve A represents the potassium-tetramethyl ammonium system for a sulfonic resin containing 23 per cent divinylbenzene.[18] Curve B is that of the potassium-lithium system for a carboxylic resin of 15.4 per cent divinylbenzene,[3, 16] while curve C shows the potassium-sodium system for the phosphonic resin, Nalcite X-219· In all three cases, the decrease in the mole fraction of a cation in the resin phase at equilibrium results in a greater resin affinity for that cation.

This change in K_D with X_i may be due to either (or both) of two effects. Either there is a change in the $(\gamma_1/\gamma_2)_i$ ratio as X_i^K varies, or else the osmotic pressure of the system changes. Reichenberg, Pepper and McCarthy[30] have shown that the increase in K_H^{Na} for the hydrogen-sodium system with sulfonic resins may be explained by using the tables for the ratios of $\gamma H/\gamma Na$ in sodium chloride-hydrochloric acid mixtures as published by Harned and Owen[21] and Robinson and Stokes.[33] These activity coefficient ratios are shown in TABLE 4. Using the equation $K_H^{Na} = \left(\frac{\gamma_{HR}}{\gamma_{NaR}}\right)$, and assuming that the ratio $\frac{\gamma_{HR}}{\gamma_{NaR}}$ shows similar qualitative features to the ratio $\gamma H/\gamma Na$ for mixed chlorides at concentrations comparable to those found in resins of various degrees of crosslinking (1.5 to 8 molal), it is seen that since $\gamma H/\gamma Na$ increases with increasing X_{Na} at these concentrations, the value of K_H^{Na} would also increase.

The relationship of the osmotic pressure to the change of K_D with X_i can be seen from the thermodynamic expression of Gregor, EQUATION 4. For the tetramethylammonium-potassium system shown in FIGURE 9, $v_{Me_4N^+} > v_{K^+}$, and therefore as X_i^K increases, the resin contracts and π decreases. Assuming that $v_{Me_4N^+}$ and v_{K^+}, remain roughly constant and independent of X_i^K then $K_{Me_4N}^K$ will decrease. Thus both activity coefficient ratios and pressure-volume changes affect K_D as X_i changes.

Selectivity as a Function of Resin Capacity

Deuel[11] has prepared a series of polyacrylic resins containing 12 per cent DVB which have different capacities. Comparing resins with capacities of 1.8 and 3.5 meq./gm., he found that the selectivity for ammonium over sodium increased with increasing resin capacity. This effect may be due to a decrease in the activity coefficient ratio $(\gamma NH_4^+/\gamma Na^+)_i$ as the molality increases. No data is available on the activity coefficients of concentrated ammonium acetate solutions. It would be of interest to have experiments of this type carried out for sodium and potassium, where the known activity coefficient of the acetates could be compared with selectivity values.

Ionic Strength of External Solution

Gregor and Bregman[18] have studied the effect of external ionic strength upon K_D for sulfonated polystyrene resins containing 2, 10, and 23 per cent divinylbenzene as a crosslinking agent. The potassium-hydrogen and potassium-lithium systems were investigated. For the 2 per cent DVB resin, no change in K_D was found for either system as the total ionic strength increased from 0.001 to 1.0. In the cases of the 10 and 23 per cent DVB resins for the potassium-hydrogen system and the 10 per cent DVB resin for the potassium-lithium system, the value of $K_{\mathrm{M}}^{\mathrm{K}}$ remained constant at ionic strengths from 0.001 to 0.1 but dropped significantly from 0.1 to 1.0. This drop can be ascribed either to activity effects or to the establishment of a counter Donnan osmotic

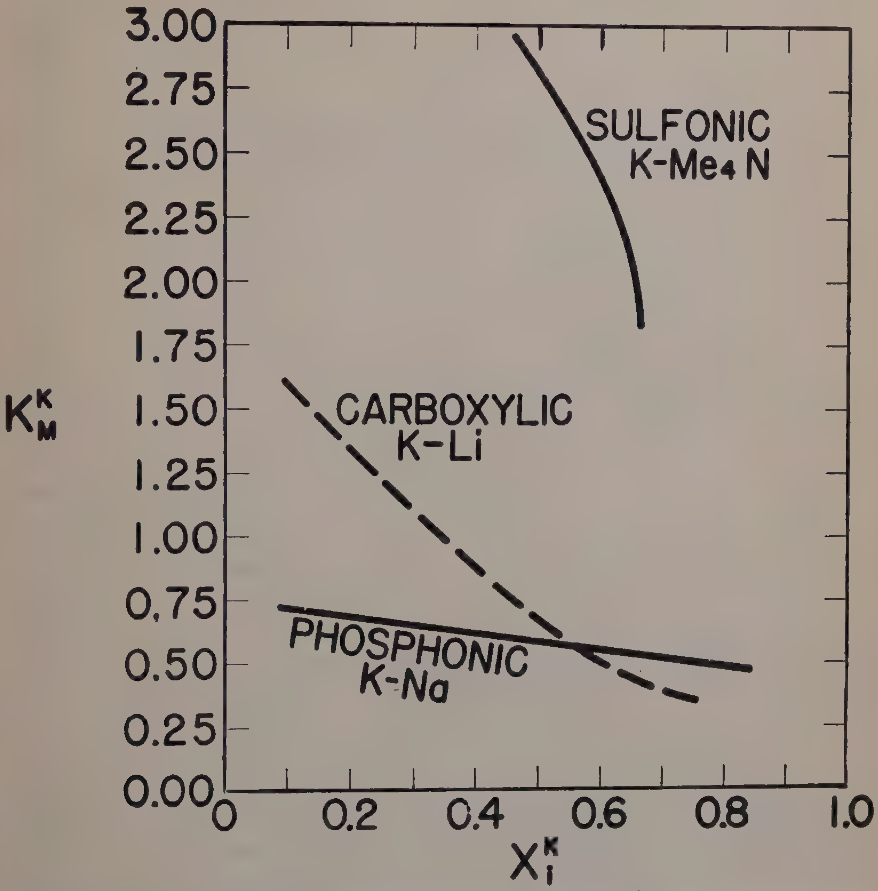

FIGURE 9. Selectivity as a function of $X_i^{K^+}$.[18] Sulfonic resin, 23% DVB, $\overset{+}{K}_{vs}$ $(CH_3)_4N^+$; carboxylic resin, 15.4% DVB, $\overset{+}{K}_{vs}$ Li^+; phosphonic resin, $\overset{+}{K}_{vs}$ Na^+.

pressure which reduces the free energy of the swelling term in the thermodynamic expression of EQUATION 4.

Temperature Effects

FIGURE 10[18] shows that for the potassium-lithium system the value of K^{K}_{Li} decreases with increasing temperature. The same type of decrease has also been described[10, 18] for the potassium-hydrogen system. In both of these cases the relatively unhydrated potassium cation is being compared with a heavily hydrated cation. Presumably as the temperature increases, the counter-ions, apart from an increased thermal motion, are partially dehydrated and, in addition, the structure of the exchanger becomes more open.[40] Similarly, Jaeger[24] in a study of the effect of temperature on ultramarine found that at 130°C., the order of selectivity reversed itself and became Li > Na > K.

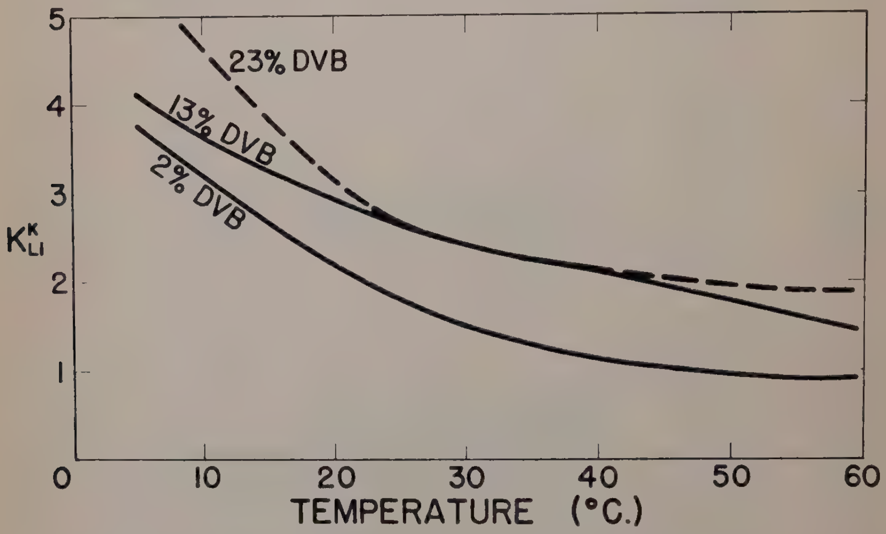

FIGURE 10. Variation of K^{K}_{Li} with temperature for sulfonated polystyrene resins.[18]

When comparing two cations H^+ and Li^+, both of which are heavily hydrated and have about the same resin volume, Gregor and Bregman[18] found that there was very little change in selectivity with temperature. A comparison of the potassium-tetramethylammonium system, where both cations are relatively unhydrated, but differ considerably in size, showed that the selectivity for potassium decreased by about 30 per cent as the temperature went from 5° to 60°C. This decrease is of the order that would be expected from the fact that the resin becomes more open and the thermal motion of the cations increases.

Summary

1. It has been shown that the nature and number of acidic groups present in cation exchange resins can be determined by titration curves.

2. The effect on the selectivity of cation exchange resins for monovalent ions of the following variables has been discussed:

A.) Structure of the resin;
B.) Nature of the acidic functional group;
C.) Mole fraction of the exchanging cations in the resin phase;
D.) Resin capacity;
E.) External ionic strength;
F.) Temperature.

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ANION EXCHANGE PROCESSES

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Obviously, anion exchange differs from cation exchange principally in that anions rather than cations are exchanged between a resinous exchanger and a solution, usually aqueous. This means that cationic groups must be incorporated into a high molecular weight organic structure to make an anion exchange resin. The only such groups which appear to have been successfully used are substituted ammonium groups. Unless these groups are quaternary, they are conjugates of weak bases and lose their ionic character in basic media. Resins whose functional groups are these primary, secondary, and tertiary amino groups are known as "weak base resins," and can exchange anions only with acid media. The quaternary ammonium resins, known as "strong base resins" are ionic in both acid and basic media, analogous to the sulfonic acid cation exchange resins.

The difference between these two types is best illustrated by the potentiometric titration curves obtained from the free base forms of the resins with strong acid. Upon addition of acid solution to a suspension of a strong base resin, the pH of the suspension decreases gradually until the acid added is nearly equivalent to the resin. Then, just as in the titration of a soluble base, the pH decreases abruptly as free acid builds up in the solution. Since the hydroxide ions of the base are in the insoluble resin and the pH is measured in the solution, to actually get this titration behavior it is necessary to add neutral salt to the solution to displace (by anion exchange) some of the hydroxide ions from the resin. Typical titration curves of this type are given by Kunin and coworkers.[1, 2, 3] Wheaton and Bauman[4] show similar titrations which use rapid stirring and high subdivision of the resin rather than addition of neutral salt to obtain contact between hydroxide ions and the electrode. Titration of weak base resins shows only a gradual increase of pH with addition of acid, since complete neutralization of the resin does not take place until a high concentration of acid is present in the solution. Thus, determination of the capacity of a weak base resin is not possible by direct titration with acid. Determination of the capacity can be accomplished[5] by exchanging the resin chloride with sodium sulfate and titrating the effluent with standard silver nitrate solution.

The principal use of anion exchange resins is the removal from water and other fluids of acids by the free base forms of the resins. Many other uses, however, have been found for anion exchange processes, analogous to the many uses of cation exchange resins.

Just as cation exchange resins are useful in the separation of cations which differ in distribution between a resin and a solution, anions may also be separated by similar techniques using anion exchange resins. While strong base resins are generally preferred for this application, Myers, Eastes, and Urquhart[6] demonstrated the separation of chloride from sulfate using a weak base resin. A strong base resin was used by Atteberry and Boyd[7] to separate perrhenate from pertechnetate and the halide ions from each other. Also separated by strong base resins are acids of biochemical interest, such as the mononucleotides by Cohn[8] and uronic acids by Khym and Doherty.[9] Since commercial strong base resins are hard to free completely of chloride ion, Berne[10] used silver oxide supported on diatomaceous earth to separate bromide, bromate, and iodide.

A great deal of interest in anion separations is not in the separation of the anions ordinarily encountered in aqueous solution, but rather of substances which can be reversibly converted to anions with proper reagents. Thus, the separations depend not only upon differences in affinities of ions for the resin, but also upon the differences in stability of the anions being separated. Examples of separations of this type are those of organic substances as anionic derivatives and metals as complex anions. Examples of organic separations are that of sugars as their borate complexes by Khym and Zill[11] and that of carbonyl compounds as bisulfite addition compounds by Gabrielson and Samuelson.[12] The latter investigators have demonstrated the separation of furfural from acetone by absorbing these compounds on the bisulfite of a strong base resin and eluting with bicarbonate. Similarly, Samuelson and Westlin[13] removed carbonyl-bisulfite compounds from sulfite waste liquors with a weak base resin, and Zager and Doody[14] removed glycerol from water by strong base resin borates.

A great deal of the effort at anion exchange separation of metals is directed at those metals, zirconium, hafnium, niobium, tantalum, and protactinium, whose cations are extremely difficult to keep in solution in simple form. Since zirconium and hafnium are so similar, their separation is of considerable interest. While these elements can be separated by cation exchange[15, 16] the method requires great care to obtain exchangeable ions. Since both elements form complex anions with fluoride, much simpler techniques have been developed by Kraus and Moore[17] and Huffman and Lilly[18] for separating them on anion exchange columns with hydrofluoric-hydrochloric acid mixtures. Similar systems have also been used to separate zirconium from niobium,[19, 20] niobium from tantalum,[21] protactinium from tantalum[22] and the niobium-tantalum-protactinium triad.[23] Other elements forming anionic complexes can be separated by this technique, and Kraus and Moore[24] have demonstrated a separation of manganese, iron, cobalt, nickel, copper, and zinc by sorption on a column of strong base resin chloride from concentrated hydrochloric acid and elution with successively lower concentrations of hydrochloric acid.

Other applications of anion exchange lie in the field of analytical chemistry, of which only a few examples will be given here. Steinbach and Freiser[25] prepared carbonate-free standard sodium hydroxide solution by passing a standard sodium chloride solution through a column of the hydroxide form of a strong base resin. Gabrielson and Samuelson[26] determined potassium in solutions containing sulfate and phosphate by passing the solutions through a column of the chloride of a strong base resin, evaporating the effluent, and weighing the potassium chloride. Samuelson and Schramm[27] use the hydroxide of a strong base resin to determine total salt concentration. The solution is passed through a column and the effluent titrated with acid. This method is slower than the analogous method using cation exchange resins, and is recommended only for solutions for which the acid of the cation exchanger interferes, such as alkali vanadates, sulfites, and phosphates.

Analogous to the use of cation exchangers as acid catalysts is the use of anion exchangers as basic catalysts. This use has not been as widespread, although it was demonstrated in 1946 by Jenny[28] for the mutarotation of glucose and in 1948 by Galat[29] in the hydrolysis of nicotinonitrile to the amide. Anion exchange resins were found by Bodamer and Kunin[30] to diminish the catalysis by cation exchange resins of sucrose inversion.

Since ion exchange resins afford a means of measuring distribution of ionic species between phases, an important application of ion exchange in pure chemistry is in the study of solution equilibria involving the exchanged ions. This method has been used extensively by Schubert and coworkers[31] with cation exchangers to study complex ion equilibria. With anion exchange resins the greatest use of this nature has been the study of column behavior of metals in solutions containing complexes. To a large extent this work has been carried out as a part of the development of separations techniques, but it often gives information on the nature of anionic species. The experiments measure in some way the distribution of a metal between the solution and the resin. This actually measures a combination of the stability of the complex and the affinity of the complex for the resin. Most of the data are presented as distribution ratios of the metal between the resin and the solution, although the "elution constant" introduced by Moore and Kraus[32] appears a better characterization of the anion exchange behavior of a metal. This function is defined by $E = Ad/V$ where d is the distance in cm. an absorption band moves when V ml. of eleunt pass through a column of cross sectional area A cm.^{2}

Elution constants are reported by Kraus and Moore for iron (III),[32] Cobalt (II) and Nickel (II),[33] and manganese (II), iron (II), copper (II), and zinc (II)[24] as a function of hydrochloric acid concentration. From their measurements, the order of the stability of anionic complexes of the +2 transition metals is found to be Zn > Cu > Co > Fe, Mn > Ni with nickel not forming anionic complexes at all. Iron is more strongly sorbed than manganese, although manganese reaches its peak sorption at a lower hydrochloric acid concentration than iron. These workers have also measured elution constants as functions of hydrofluoric and hydrochloric acid concentrations for zirconium and niobium,[19] tantalum,[34] and protactinium.[23] Distribution data between solution and resin are given by Kraus and Moore[35] for protactinium in hydrochloric acid, and Huffman and Lilly[18] for zirconium and hafnium in hydrofluoric, hydrochloric acid mixtures, and by Huffman, Iddings, and Lilly[20] for zirconium, hafnium, niobium, and tantalum in hydrochloric acid. Many of these workers discuss the possible formulas of the anions sorbed. Kraus and Moore[23, 24] believe that the anions most strongly sorbed from fairly concentrated acid are the singly charged species. Huffman and Iddings[36] have attributed a multiple peak elution of niobium to slow attainment of equilibrium between different ionic species.

Other uses of anion exchange in the study of complexes are the study by Salmon[37] of ferric phosphate complexes and removal of iron (III) from solution by the phosphate of a strong base resin, the investigation by Samuelson[38] and

Teichert and Rinman[39] using weak base resins into the nature of certain polyphosphates, and the investigation by Leden[40] into the complexes of copper and cadmium. Using columns of a strong base resin with the appropriate anion, the latter investigator found anionic complexes absent in solutions of cadmium perchlorate, copper perchlorate, and cadmium sulfate but present in solutions of copper sulfate, chloride, and acetate and cadmium chloride and iodide.

The measurement of rates, equilibria, and thermodynamics of anion exchange processes lags behind that of cation exchange processes. Except for the recent work of Soldano and Boyd,[41] the very few studies of anion exchange seem limited to processes involving weak base resins, although the exchange processes of strong base resins are known to be quite rapid. Nachod and Wood[42] use second order kinetics to fit the hydrochloric acid sorption by two different weak base resins and Bishop[43] shows graphically the rates of uptake of several acids by another weak base resin. Kunin and Myers[44] have studied effects of particle size, hydration, concentration, amount exchanged, stirring, interruption, and added salt on processes involving a similar resin and have summarized the work in their book.[1]

Equilibria involving anion exchange resins have been less neglected than rates, but not nearly as extensively studied as cation exchange equilibria. Wheaton and Bauman[4] list, for a large number of singly charged anions and two strong base resins, equilibrium "constants" defined for one concentration by

$$K = \frac{(A^-)_R(Cl^-)_S}{(A^-)_S(Cl^-)_R} \tag{1}$$

where the subscripts R and S refer to concentrations in the resin and solutions, respectively. They also give equilibrium diagrams for a few of the exchange reactions over a range of concentrations and proportions. Kunin and McGarvey[2] also measured equilibria involving several salts with a strong base resin, fitting it graphically by the equation

$$\frac{(OH^-)_S}{(OH^-)_R} = K_c\left(\frac{(A^-)_S}{(A^-)_R}\right)^p \tag{2}$$

Gregor and Belle[45] have made a thorough study of the properties of a strong base resin, including the effect of concentration and crosslinking on exchange equilibria of a wide variety of anions. The constants of EQUATIONS 1 and 2 are listed in TABLE 1 for a number of anions. It is to be noted that the values of K are for equilibrium with chloride, those for K_c with hydroxide. Since the value of K depends on the resin anion composition, the mole fraction, X_{Cl}, of chloride in the resin is tabulated for each K. Noteworthy is the high affinity of the resins for large organic ions and the low affinity of Dowex-1 for hydroxide. The effect of crosslinking in the resin on exchange equilibria was demonstrated by Wheaton and Bauman[4] and Kunin and Myers.[46]

Much more extensive are the equilibrium data on weak base resins, especially dealing with acid sorption by the free base. Since the measurement is of acid leaving solution and being picked up by the resin, the results are usually expressed as "adsorption isotherms." Myers, Eastes, and Urquhart[6] use the

Freundlich isotherm to describe sorption of hydrochloric and sulfuric acids, and Jenny[28] has measured nitric acid sorption. Kunin and Myers[47] present graphically sorption of a wide variety of acids as a function of solution pH and also show exchange equilibria for several anions with the chloride of the resin. TABLE 1 includes the per cent exchanged for certain anions when equal quantities of the two ions are in the system. Robinson and Mills[48] report the sorption of a series of straight chain aliphatic acids from water, acetone, and liquid hydrocarbon and show the effect of chain length on the equilibrium.

TABLE 1

SUMMARY OF ANION EXCHANGE EQUILIBRIUM MEASUREMENTS

Resin, Constants	Dowex-1[4]		Dowex-2[4]		Dowex-2[45]		IRA-400[3]		IR-4B[47] % Exch.
	K	X_{Cl}	K	X_{Cl}	K	X_{Cl}	K_0	P	
β-naphthalene sulfonate					67	0.01			
Dichlorophenate			53	0.21					
Salicylate	32.2	0.18	28	0.16					
Perchlorate					32	0.04			
Thiocyanate					18.5	0.05	6.3	0.65	
Trichloroacetate					18.2	0.05			
p-toluenesulfonate					13.7	0.07			
Iodide	8.7	.27	7.3	0.27	13.2	0.07	9.0	0.55	
Phenoxide	5.2	.34	8.7	0.26					
Bisulfate	4.1	.29	6.1	0.48					
Benzene sulfonate					4.0	.20			
Nitrate	3.8	.38	3.3	.36	3.3	.24	8.5	0.69	62
Bromide	2.8	0.40	2.3	.48	3.4	.22	6.8	0.58	
Trifluoroacetate					3.1	.26			
Dichloroacetate					2.3	0.34			
Nitrite	1.2	.51	1.3	.52					
Bisulfite	1.3	.48	1.3	.42					
Cyanide	1.6	.47	1.3	.56					
Chloride	1.00	—	1.00	—	1.00	—	2.95	0.75	50
Bisilicate			1.13	.73					
Bromate			1.01	.52					
Hydroxide	0.09	.77	0.65	.56	0.69	.59	1.00	—	
Bicarbonate	0.32	.65	0.53	.63					
Dihydrogen phosphate	0.25	.68	0.34	.63					
Monochloracetate					0.21	0.88			
Iodate					0.21	.83			
Formate	0.22	.70	0.22	.68			1.8	1.45	
Acetate	0.17	.73	0.18	.70	0.17	.86	0.8	0.49	46
Fluoride	0.09	.77	0.13	.74	0.104	.90	1.15	0.94	46
Aminoacetate	0.10	.77	0.10	.76					

Peterson and Jeffers[49] have studied a series of aliphatic acids of a range of strengths and find the sorption appears to be governed partially by acid strengths at low concentrations, but principally by the size of the molecule at high concentrations. TABLE 2 illustrates this by giving the sorption at rounded values of concentration. These results could not be described by the Freundlich isotherm and are described only poorly by the Langmuir isotherm.

Peterson and Gowen[50] have measured sorption of a series of aromatic acids by two different weak base resins. These results show very little correlation with the other properties of the acids. For example, FIGURE 1 shows isotherms for benzoic acid and a series of meta-substituted acids of different strengths with the resin Amberlite IR-4B. While one would expect the attraction of a basic resin for an acid to be closely related to the acid strength, the figure shows acids both stronger and weaker than benzoic, all much more strongly adsorbed

TABLE 2

SORPTION (MILLIMOLES/G.) ON AMBERLITE IR-4B AT ROUND EQUILIBRIUM MOLARITIES OF ALIPHATIC ACIDS[49]

Acid	$K_a \times 10^5$	$c = 0.1$	$c = 0.3$	$c = 0.6$	$c = 1.0$
Chloracetic	137.8	5.9	6.8	7.5	
Formic	17.12	6.0	7.0	7.3	8.4
Acetic	1.754	4.3	5.2	5.6	6.3
Propionic	1.34	4.2	5.1	6.0	7.3
Butyric	1.50	4.6	6.5	9.3	
Isobutyric	1.38	4.1	5.9	8.2	

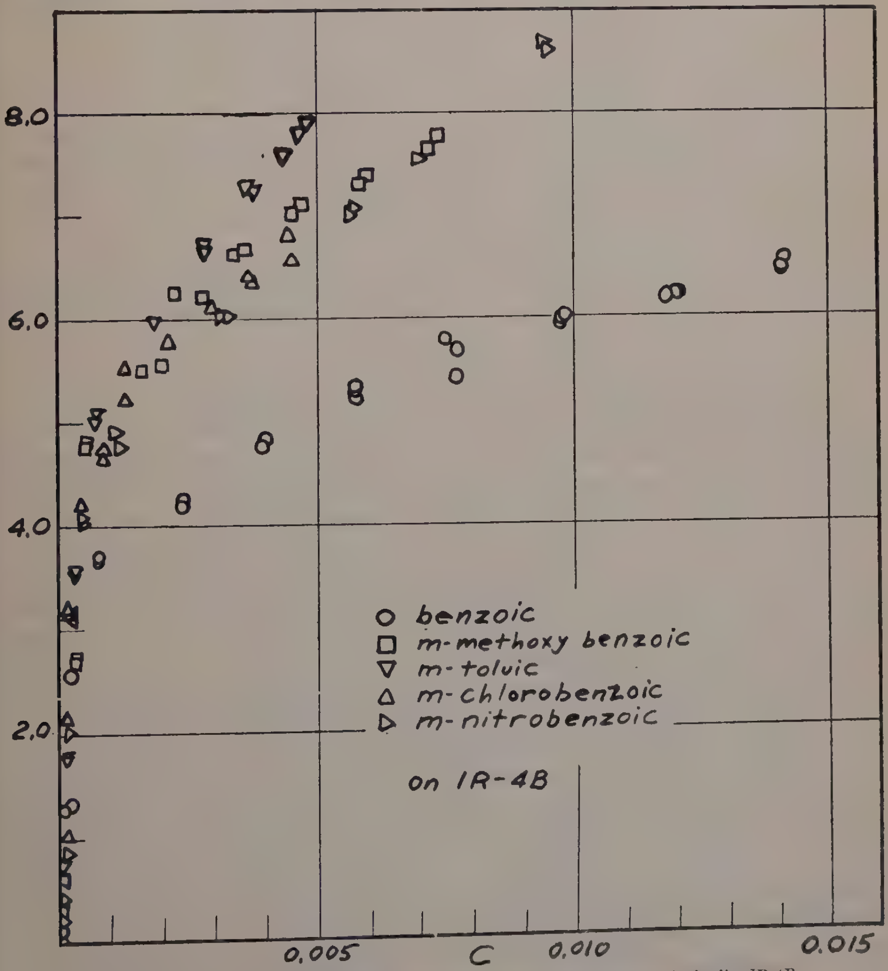

FIGURE 1. Sorption of benzoic acid and meta-substituted benzoic acids by Amberlite IR 4B.

by the resin than benzoic, with the weakest of the group, m-toluic, most strongly sorbed. FIGURE 2 shows the aminobenzoic acids to be somewhat more strongly sorbed by the same resin than benzoic and much more so than acetic which is more nearly the same strength acid. This may be due to an interaction between the amino groups of the acids and the phenolic groups known to be in the resin, since the non-phenolic resin, Amberlite IR-45, sorbs these acids less than benzoic, as seen in FIGURE 3. On the other hand, the much stronger nitro acids are seen in FIGURE 4 to be less strongly sorbed by this latter resin than the methoxybenzoic and metatoluic acids, which are not nearly as strong, while the ortho-nitro acid, the strongest acid of the group, is even less strongly sorbed than benzoic. Bishop[43] has suggested that acids of greatly differing strengths may be compared, if no common ion is present, by one logarithmic graph for all acids of sorption as a function of hydrogen ion concentration. This is not adequate, however, for the aromatic acids, since, as seen above, the sorption isotherms are not in the order of acid strengths. It can be seen in FIGURES 5 and 6, which show the sorption of several of the acids plotted against hydrogen ion concentration, that little if any correlation is obtained.

If the acid is being sorbed from a solution containing a common ion, Bishop[43] recommends expressing the equilibrium in terms of the product of concentrations (or better, activities) of the two ions of the acid. This method he has applied with some success[51] to the uptake of acetic, chloracetic, and hydrochloric acid by the hydroxide of a strong base resin. This was found inadequate, however, by Davies and Jones[52] for high concentrations of hydrochloric acid with a weak base resin. These investigators used analysis for both ions of the acid in acid-salt solutions equilibrated with the resin to determine the water absorption which, under the conditions of their experiment, had to be considered to obtain accurate values of the acid sorption.

In a study of the uptake of aliphatic acids by the hydroxide of a strong base resin, Peterson and Jeffers[53] find that the removal of acids from solutions of high concentration is considerably greater than the capacity of the resin and must be accounted for by some means of sorption, other than neutralization, by the hydroxide ions in the resin. This has been called "adsorption," but because of the small surface of the resin it is more probably a distribution between the aqueous solutions inside and outside the resin granules. A similar behavior of weak bases with cation exchange resins has been reported by Davies and Thomas[54] along with measurements of sorption of carboxylic acids by *cation* exchange resins. A similar behavior of salts was found by Lowen and coworkers[55] in measurements of cation exchange equilibria.

This type of distribution has attracted the attention of a number of investigators. Kraus and Moore[56] have studied the sorption of hydrochloric acid by the chloride of the strong base resin, Dowex-1, with acid molalities from 0.002 to 16, and they have calculated equilibrium constants for the acid in the resin. Katzin and Gebert[57] have found that strong base resin salts strongly absorb inorganic salts from acetone solution. Not only are salts like cupric or cobalt chloride, which might be expected to form complex anions, sorbed, but also lithium salts and cobalt and nickel nitrates are removed from acetone even by the resin nitrate. Increasing the water content of the resin favors the sorp-

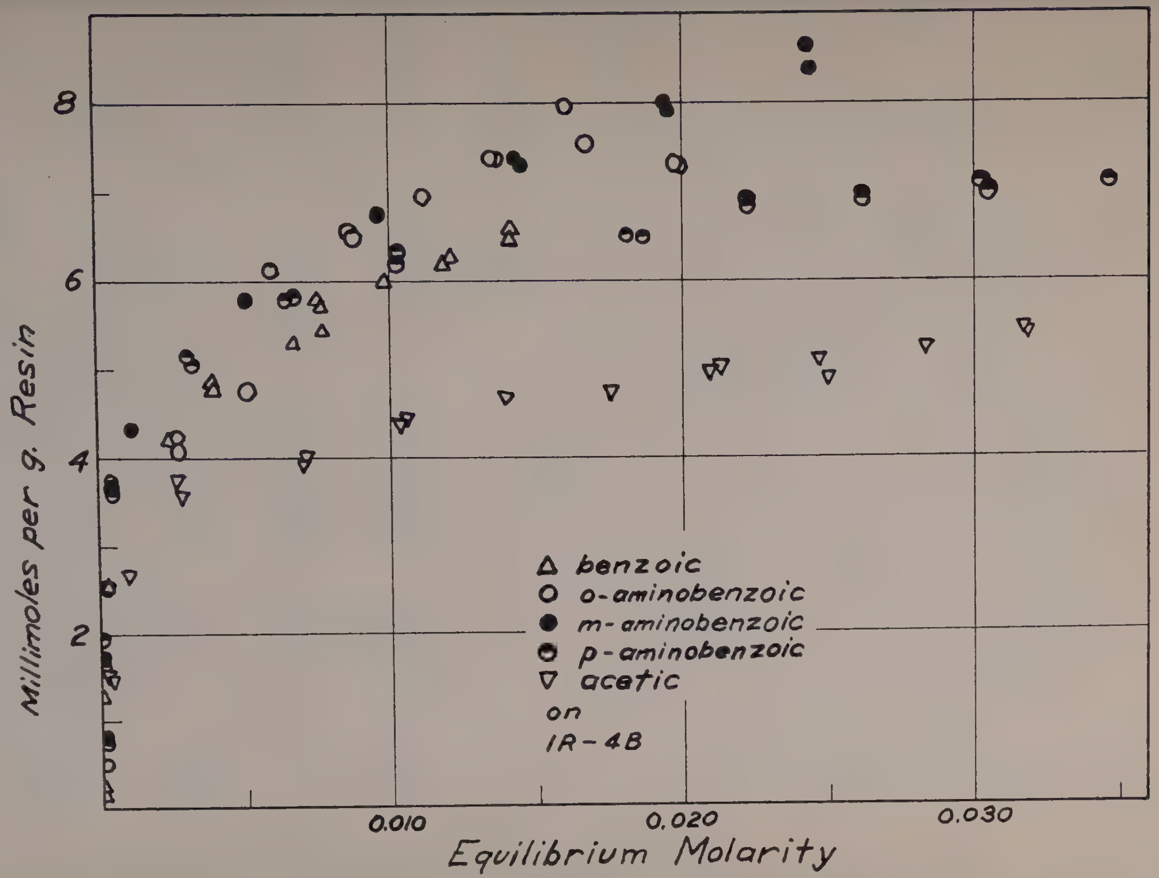

FIGURE 2. Sorption of benzoic acid, acetic acid, and the aminobenzoic acids by Amberlite IR-4B.

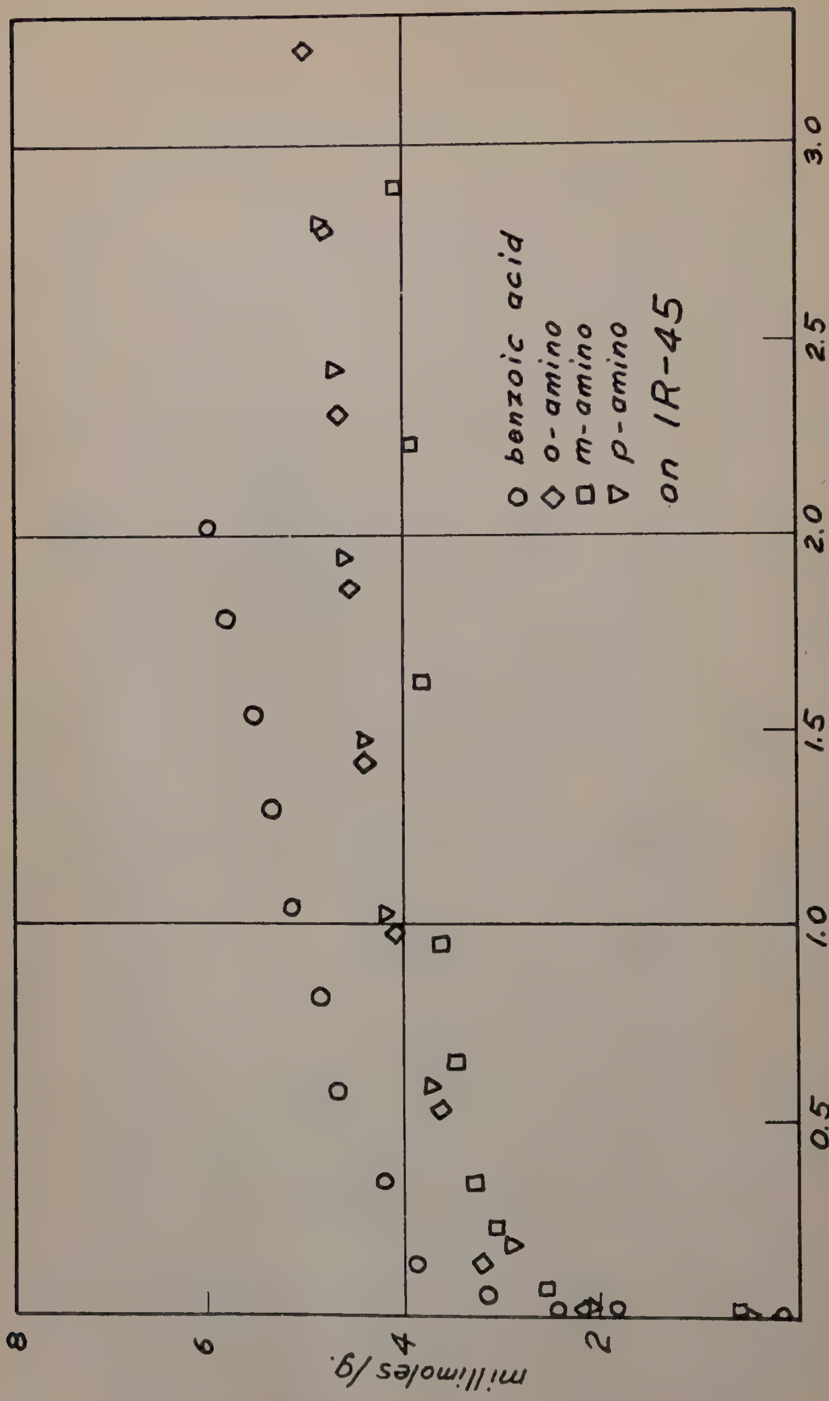

FIGURE 3. Sorption of benzoic acid and the aminobenzoic acids by Amberlite IR-45.

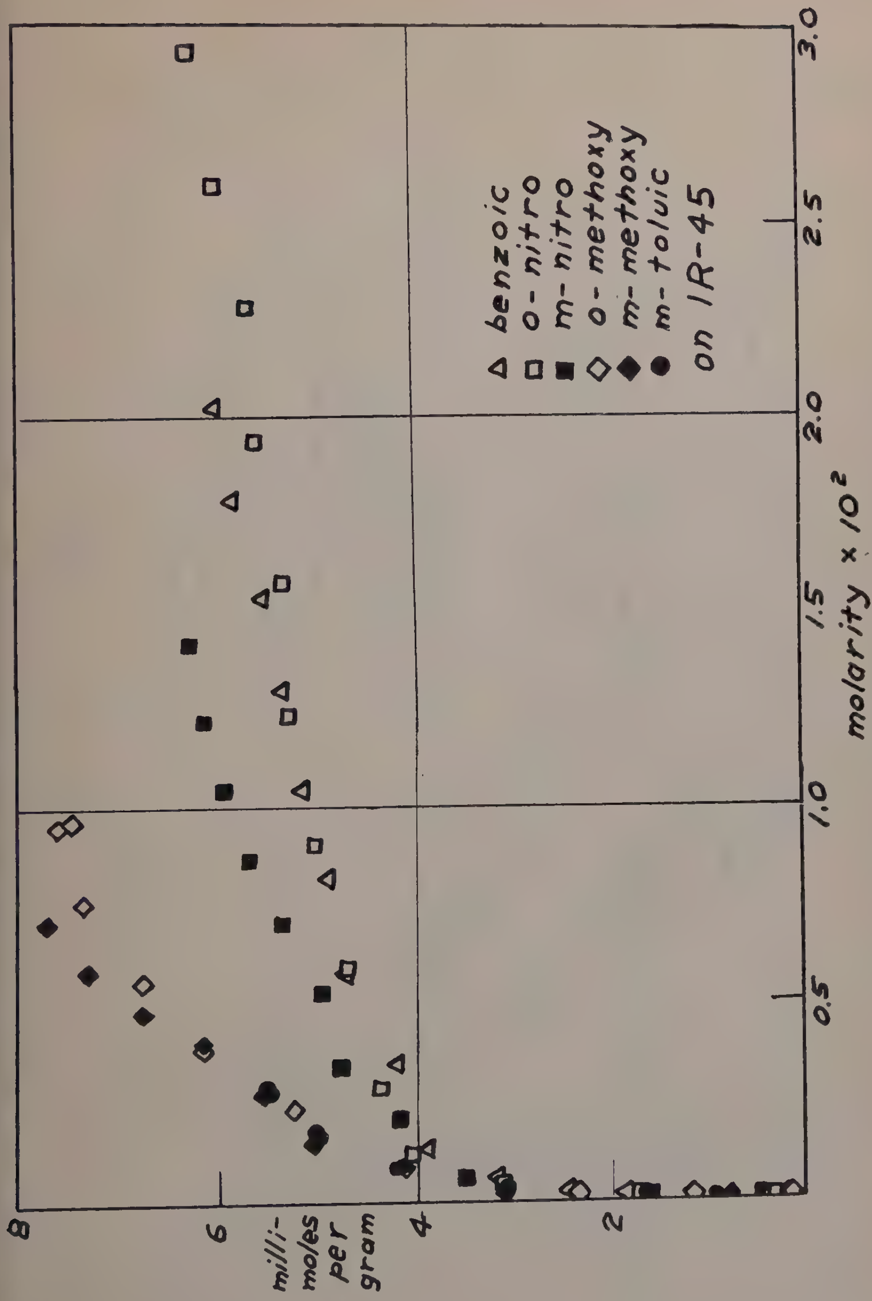

Figure 4. Sorption of benzoic acid and substituted benzoic acids by Amberlite IR-45.

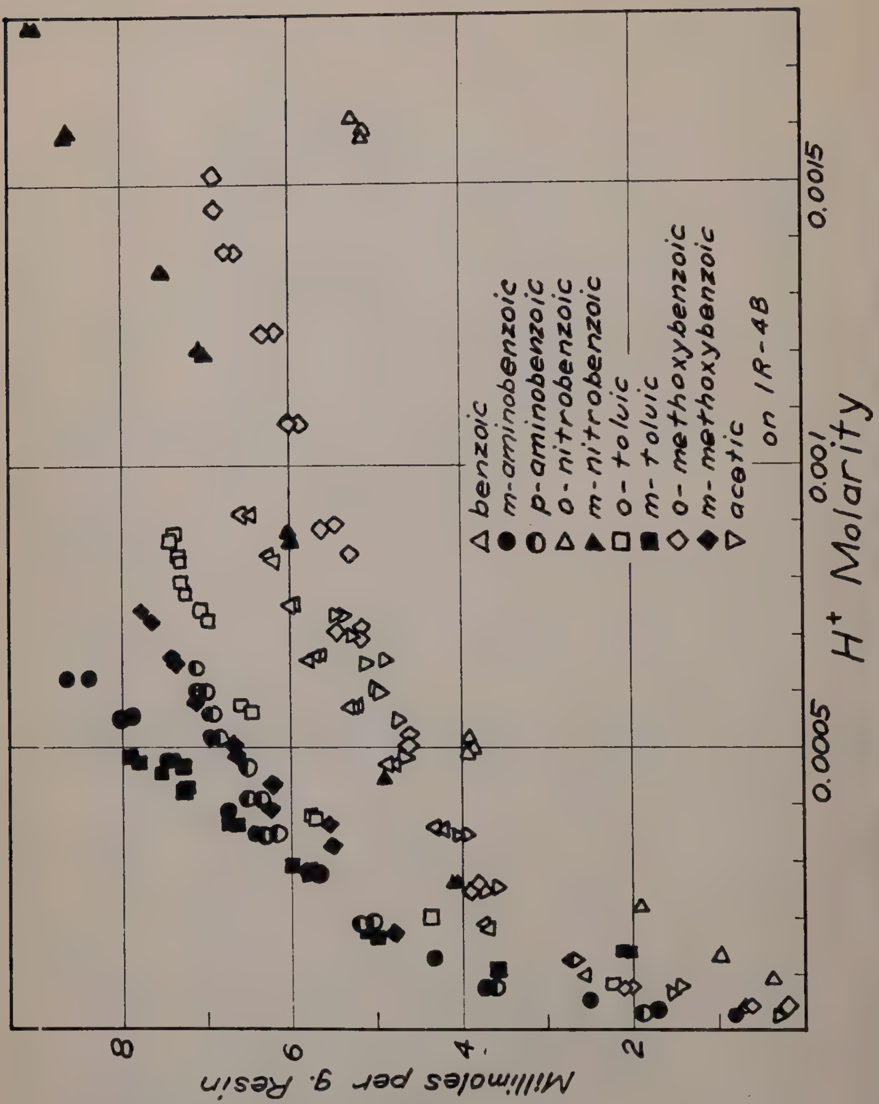

FIGURE 5. Sorption of acids by Amberlite IR-4B plotted against hydrogen ion concentration.

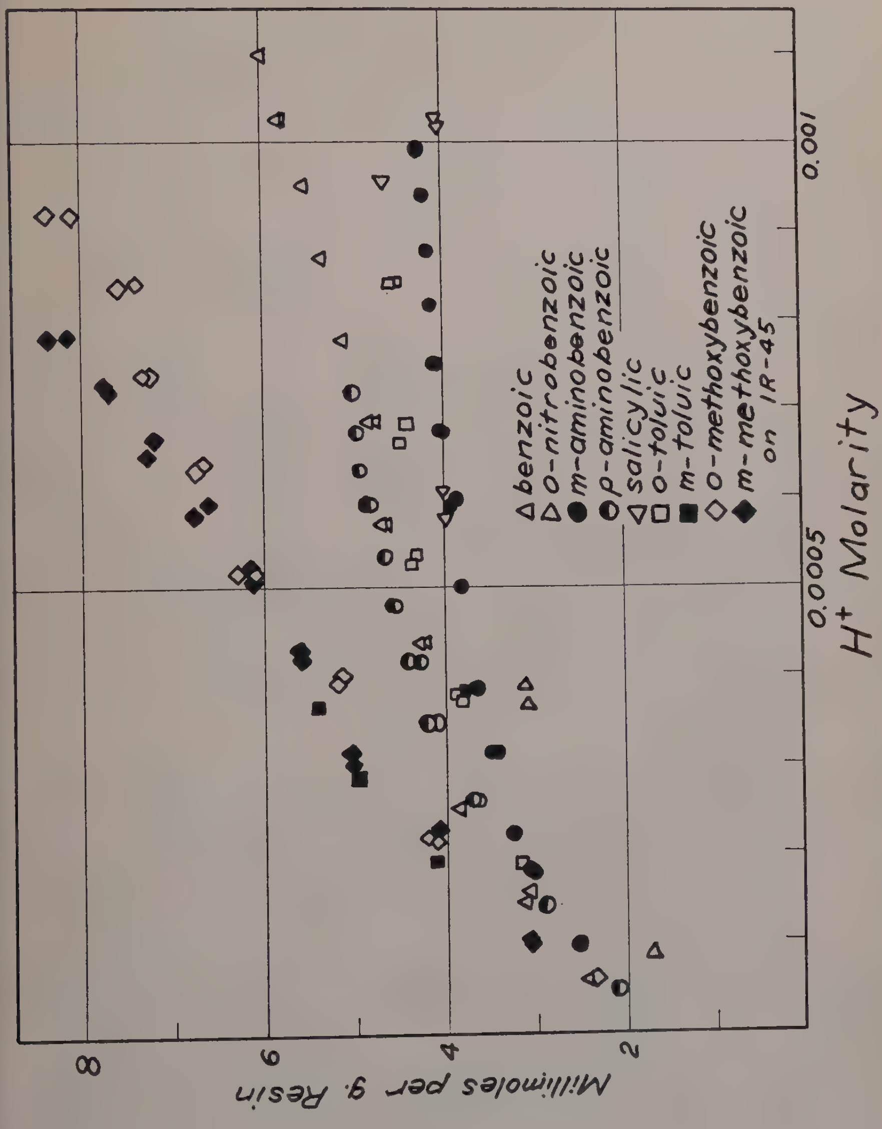

FIGURE 6. Sorption of acids by Amberlite IR-45 plotted against hydrogen ion concentration.

tion, showing that the water in the resin is needed to accommodate the salt. Wheaton and Bauman[58] have used the distribution of unionized molecules between resin and solution in ingenious column separations of these substances from strong electrolytes and from each other.

Sorption from aqueous solution of aliphatic acids has been studied by Peterson and Footerman[59] using the chlorides of three strong base resins and the nitrate and p-toluenesulforate of one. The effect of acid nature on the sorption is shown in FIGURE 7. Just as in adsorption of acids by charcoal and as expected from the results of Peterson and Jeffers,[49, 53] the acids with the greater number of carbon atoms are more strongly sorbed. The acids are nearly equally sorbed by the resin chloride and nitrate, but significantly less so on the p-toluenesulfonate, on the basis of resin weight. This difference becomes small, however, when the comparison is based on equivalents of resin, because of the greater mass of the p-toluenesulfonate anion. It thus appears that the sorption is not due to attraction for the acid by the anion in the resin. The effect of resin structure on absorption is seen from a comparison of Dowex-1 and Dowex-2 (nalcite SAR). These resins differ only[4] in that the functional group in Dowex-1 is a trimethylammonium, that in Dowex-2 dimethyl-β-hydroxyethyl-ammonium, the fourth bond of the nitrogen in each being used to attach the group to the polymeric structure. As can be seen in TABLE 3, this makes

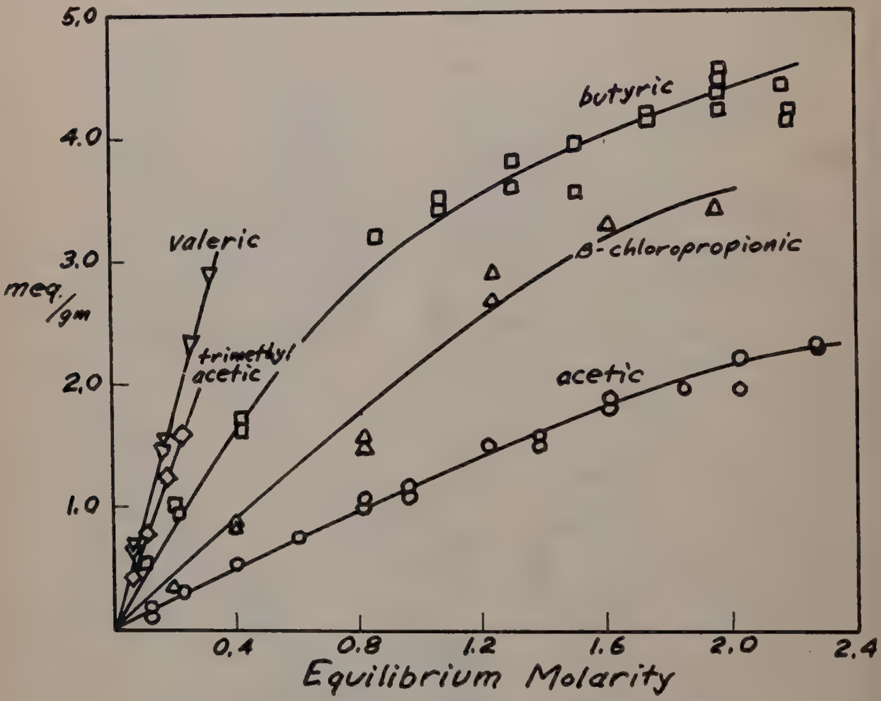

FIGURE 7. Sorption of aliphatic acids by Nalcite SAR (Dowex-2) chloride.

little difference in sorption of acetic acid, but the Dowex-1 structure appears to favor butyric acid.

From the information available it can be seen that anion exchange resins have a variety of applications, with many yet to be discovered. In addition, the studies made of the fundamental properties of the resins show that far more study is needed before the factors governing anion exchange are well understood.

TABLE 3

SORPTION BY STRONG BASE RESIN SALTS FROM 2*M* ACID SOLUTION[59]

Resin	Acetic		Butyric	
	meq/gm	*meq/eq*	*eq/gm*	*meq/eq*
Dowex-1 chloride	2.10	778	5.85	2160
Dowex-2 chloride	2.15	795	4.31	1600
Dowex-2 nitrate	2.05	815	4.50	1790
Dowex-2 p-toluenesulfonate	1.25	634	3.25	1650
IRA-400 chloride	2.30	1000	2.90	1260

I would like to express my appreciation to Oak Ridge National Laboratory for permitting me to contribute to this monograph, to the University of Louisville, in whose laboratories some of the work reported has been performed, to the Atomic Energy Commission for support of the work, to the resin manufacturers for samples used in the work, to Kurt Kraus, Leonard Katzin, and Harry Gregor for making available results which had not yet been published, and to John Wiley and Sons, Inc., and The American Chemical Society for permission to illustrate the oral version of this paper with certain copyrighted figures.

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NON-IONIC SEPARATIONS WITH ION EXCHANGE RESINS*

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Introduction

Ion exclusion has recently been described[1] as a new unit operation for the separation of ionic from non-ionic components in aqueous solution by use of ion exchange resins. It provides a means for deionization without the use of chemical regenerants, depending only on the physical properties of the resin, specifically the exclusion of the ionic material from within the resin particle.

In that work it was pointed out that certain large non-ionic molecular structures, *e.g.* sugars, could not be separated effectively from ionic materials, *e.g.* salt. In this paper we show that a wide spectrum of sorption and exclusion of various non-ionic materials by the resins actually occurs. This determines not only the degree of separation in ion exclusion but also, by use of the same techniques, makes possible the separation of two or more non-ionic components.

It is possible by fairly simple batch experiments to determine the inside-outside distribution constants for a given solute and a given resin under various conditions, and from these values predict the position of the elution curves and thus the loading of the resin bed and the degree of separation.

Ion Exclusion

Inasmuch as the present subject ties in so closely with ion exclusion, it is necessary to reconsider some of the characteristics, limitations and values of ion exclusion and especially to understand just what ion exclusion is.

Ion exclusion is a method for the separation of ionic and non-ionic materials, present in water or some other polar solvent, by the use of ion exchange resins. It involves loading of the top fraction of an immersed resin bed with a solution mixture of ionic and non-ionic fractions followed by elution of the two components with the same solvent. (Henceforth, in this discussion, we shall consider only the case of water which will be the solvent generally used elsewhere.) If properly carried out, the components will be separated into two discrete fractions, but somewhat diluted. Operations may be set up on a semi-continuous basis requiring no regenerant chemicals.

Figure 1 shows an example of a typical pilot plant run using this process. It shows the semi-continuous feed of a salt-non-ionic mixture followed in turn by water, additional feed solution, water, etc. The resin bed used in this case was 6.25 inches in diameter by 60 inches and filled with Dowex 50-X8, 50-100 mesh (X8 refers to 8 per cent crosslinkage with divinylbenzene). The feed solution was a mixture of approximately 3 per cent salt and 3 per cent non-ionic component. For each cycle, this mixture was fed in a volume equal to approximately 20 per cent of the bulk resin volume or 1.57 gallons. Three volumes of rinse water were used per volume of feed which is quite typical over a fairly wide range of concentrations. Flow-rate was maintained at 0.62 gpm/ft.[2]

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* The authors wish to thank Mr. D. F. Harrington for his assistance in obtaining data used in this report.

which meant that the cycle was repeated every 48 minutes. By usual standards this shows a very rapid development of the chromatogram. Each fraction is about equal in volume and thus the resultant concentration is about one-half that of the feed solution.

A great number of separations of highly ionized salts, acids and bases, from slightly ionized or non-ionic materials, have been carried out with similar results. Whether the fractions be organic or inorganic makes no difference, hydrochloric and boric acids fractionate essentially the same as sodium trichloroacetate and acetic acid.

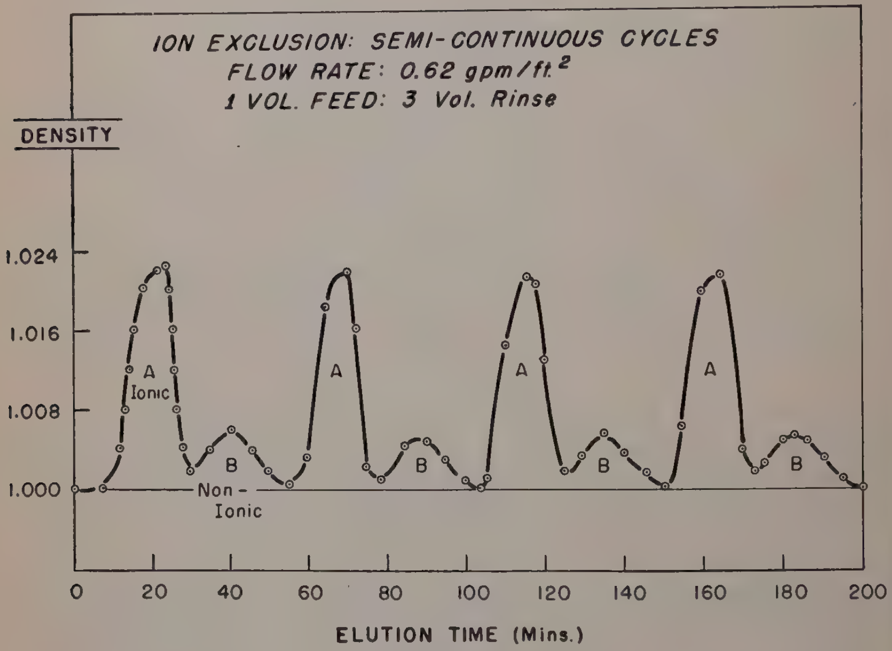

FIGURE 1.

As shown in the paper already referred to,[1] there are a number of factors, very closely interrelated, which affect the degree of separation.

Generally the volume of feed solution must be small with respect to the total bulk resin volume. Ion exclusion operates on the principle that the ionic fraction is excluded from the resin particle due to Donnan membrane effects while the non-ionic fraction shows little concentration differential between inside and outside resin phases. Without a differential the feed volume must be less theoretically than the volume of water inside the resin beads and practically much less. For example, the occluded water volume inside the resin bead is about 42 per cent of the bulk resin volume for Dowex 50–X8, 50–100 mesh as just described, while the feed volume was only 20 per cent. In some cases feed volumes as low as 5–10 per cent have to be used for the same resin. This is the case when ionic concentrations are high because exclusion of the ionic fraction decreases as concentration increases. It is interesting and very important that separations are little affected by the non-ionic concentration.

Rinse volumes must be fairly large, theoretically greater than the occluded water volume of 42 per cent. In the case just shown, we used approximately 60 per cent.

Though the best separations are possible with a high capacity highly ionized exchanger, some degree of separation is possible with a wide variety of types with the limitation that the resin be unreactive with the solute. The crosslinkage and particle size of the exchanger both are important factors in determining the degree of separation. In both cases a compromise must be made. Low crosslinked resins favor more rapid diffusion of the non-ionic fraction, but poorer exclusion of the ionic portion. Dowex 50 resins made with from 4 to 12 per cent divinylbenzene fall in the range practical for most operations. Separations improve directly with decrease in particle size but the reduction in size is limited by the increased pressure drop through the column. This importance depends to a very great extent, of course, on the end use. For analytical purposes a very slow flow might be permissible whereas industrially it would be prohibitive in cost.

Other factors affecting the quality of the elution curve are temperature and flow rate. Good separations are favored by elevated temperature and reduced flows, in each case because of the importance of approximating equilibrium at any given stage in the column.

As mentioned in the earlier work one of the first exciting possibilities of ion exclusion was that it might make possible a very economical means for purifying sugars. Attempts to put this into practice, however, met with failure because the sugar (sucrose) like the salt (sodium chloride) was apparently effectively excluded from the resin and no appreciable separation was realized. This was thought to be a very special case because of the size and configuration of the sucrose molecule. But if sucrose behaved on elution like sodium chloride, would it not be possible, then, to separate the sugar from other non-ionics exactly as the salt? That this separation was equally successful may be seen in FIGURE 2. In this case 15 ml. of 4 per cent glucose and 4 per cent methanol were eluted and separated into two fractions using a 100 ml. resin bed. The hydrogen form of Dowex 50–X8, 50–100 mesh was used in this separation. Thus, though a high degree of ionization insures exclusion of a compound from within a resin particle, there is another factor (or factors) which yields the same end result.

Experimental Fractionations

In all of the chromatographic separations described in this paper, with the exception of that shown in FIGURE 1 which was on pilot plant scale, the data were obtained using the equipment shown in FIGURE 3. The column was an 100 ml. burette filled with 100 ml. of resin. Resin bed dimensions were approximately 1.5 cm. x 55 cm. The voids between the resin particles were completely filled with water to assure no channeling through the resin bed and the water drained down to the top resin level prior to the start of each run.

The predetermined volume of feed solution was then loaded above the resin and elution commenced at the proper flow rate, generally 1 ml./min. In the plots of elution curves the effluent volume is measured from the start of loading the column. The loading is continued until the feed solution has similarly reached the top resin level after which rinsing is commenced by the introduction of water above the resin and continued until all of the components of the feed solution are eluted from the column. The column is now restored to its initial condition.

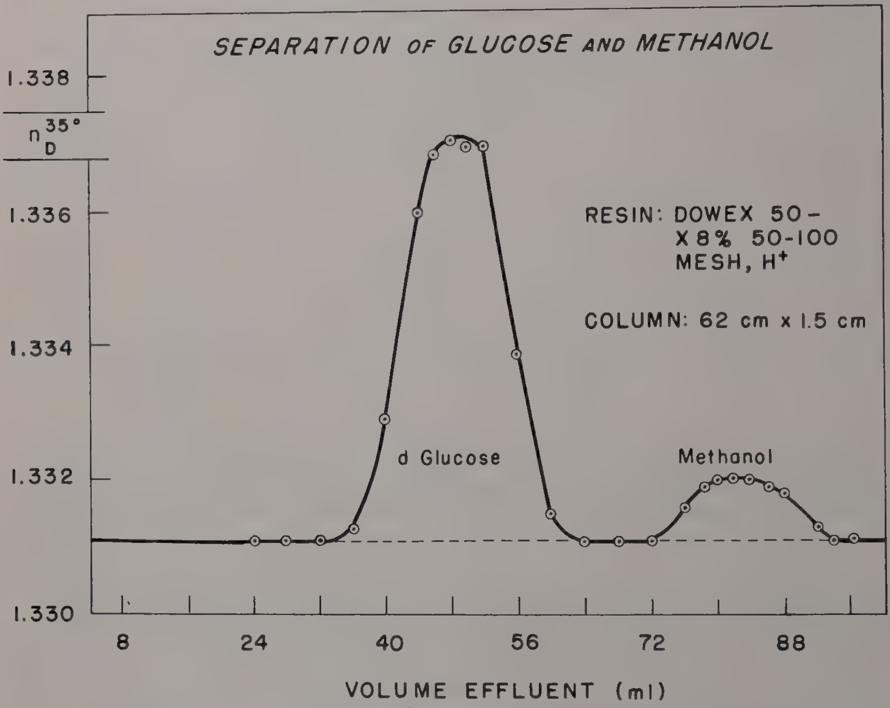

FIGURE 2.

The samples are collected in test tubes by use of a Technicon Fraction Collector which delivers a predetermined number of drops into each tube before moving on to the next. Thus a maximum of 200 cuts of from 1 to 400 drops each may be taken. The instrument has one drawback, in that individual droplets vary in size with surface tension as the quantity and kind of solute are changed. Even though a given burette tip may be calibrated with water, calibration may be notably inaccurate in some parts of the run. In some cases, it has been possible to see the elution curve by inspection of liquid volumes in the tubes. For this reason, we have more recently practiced measuring cut volumes and are now using calibrated test tubes. Analyses of the effluent may be made by any of several methods—colorimetry, titration, density—but by far the greater number of samples have been checked

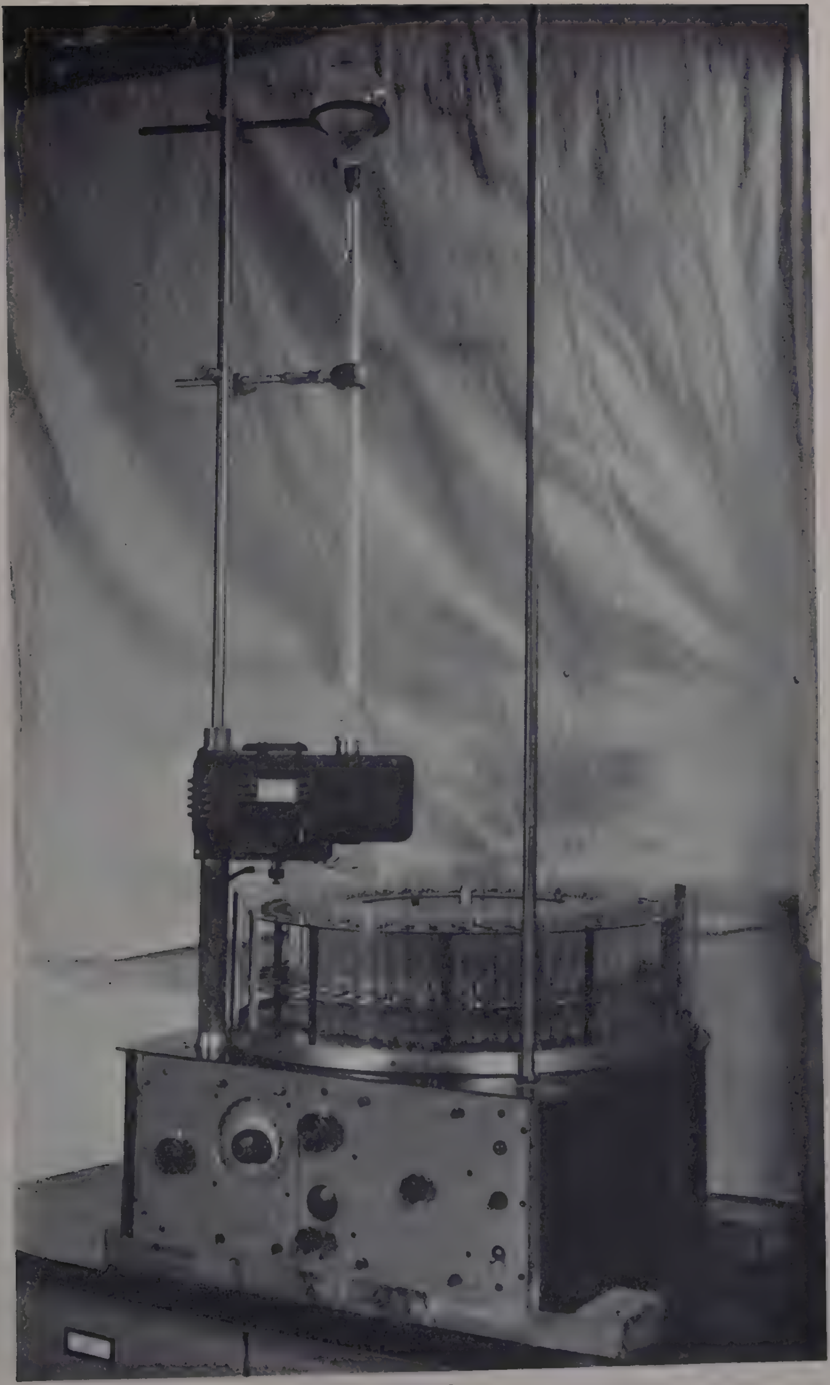

Figure 3.

using the refractometer either alone or in conjunction with one of the other methods.

Distribution Constants

As ion exclusion investigations proceeded it was realized that not all non-ionic compounds behaved exactly the same. In some cases, separations from sodium chloride were poor for a given material while another compound handled in identical fashion would separate very well. Studies were made with several families of compounds and generally the higher molecular weight members separated more poorly from salt than did the lower ones. This is clearly shown in FIGURE 4 for the separations, using Dowex 50–X8, 50–100 mesh, of sodium chloride from the polyhydric alcohols. As stated earlier, the disaccharides (sucrose) and monosaccharides (d-glucose) could not be separated from salt. Xylose (a pentose) on the other hand shows a fair degree of separation which is even more improved with pentaerythitol, glycerol, and ethylene glycol. Similar results may be seen in the case of the polyethylene glycol series, FIGURE 5, and the polyethylene amine series, FIGURE 6, to the extent they were investigated. This noted difference in behavior of different non-ionics suggested a spectrum of exclusion factors rather than lumping them all together in one category. It will be seen later that these exclusion factors do not correlate in every case with the location on the effluent curve of the component in question. For example, in the case of the polyethylene

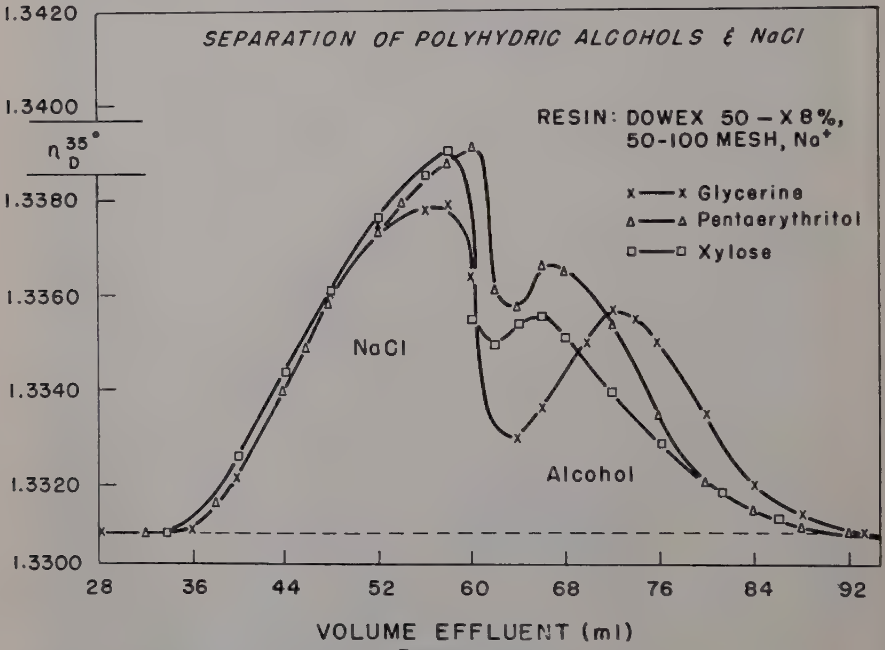

FIGURE 4.

polyamine series, the highest member appears from the elution curve to be the most strongly excluded while determination of the distribution constants (below) shows the reverse to be true by a small factor. Thus what was once believed to be strictly a case of difference in distribution at equilibrium is now found to be partially a result of equilibration rate. Actually, then, in some of these runs operations deviated considerably from true equilibrium conditions.

To determine this distribution of solute between the water inside the resin phase and that outside, it became necessary to determine distribution constants, K_d. This involves simply contacting the resin with a solution of the solute in question for a time sufficient to reach equilibrium and then to determine the concentration of each fraction. K_d is defined as the ratio of solute concentration inside the resin (C_{Si}) to that outside (C_{So}), thus $K_d = C_{Si}/C_{So}$.

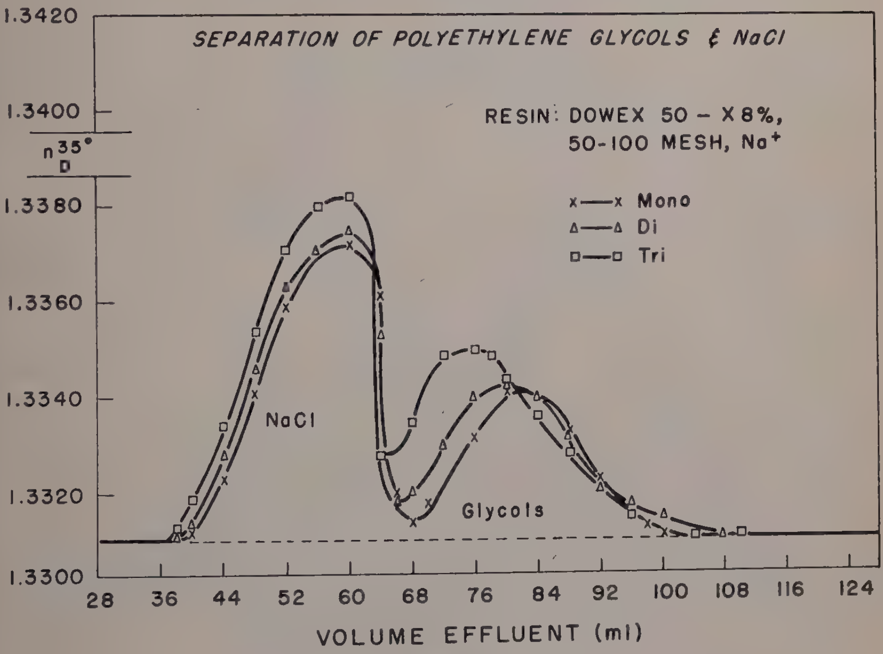

FIGURE 5.

C_{Si} and C_{So} are defined on a wt./volume relationship, *e.g.* grams/ml., thus:

$$C_{Si} = \frac{M_{Si}}{V_{Si} + V_{Wi}} \quad \text{and} \quad C_{So} = \frac{M_{So}}{V_{So} + V_{Wo}}$$

where V_{Si} = volume of solute inside the resin and V_{Wi} = volume of water inside the resin. V_{So} and V_{Wo} are similar terms for the outside portion. M_{Si} and M_{So} refer to the weight of solute in inside and outside portions. Analysis of the two phases becomes considerably more of a problem, especially in the case of organic compounds which do not readily lend themselves to direct and accurate analysis. Bauman and Eichhorn[2] in their analysis of hydrochloric acid distribution in Dowex 50 analyzed aliquot samples of the external solution by direct titration. The resin itself was then surface dried briefly with some good absorbent such as blotters and the inside solution composition checked by rinsing and titrating this rinse from one fraction while determining moisture content of another. More recently both Pepper *et al.*[3] and Gregor *et al.*[4] have adopted the procedure of centrifuging the resin sample to extract the outside solution before analyzing the resin fraction. Gregor concludes that surface blotting as well as suction-drying apparently removes some water from the resin interior. Duplication of results is very good as both Pepper and Gregor have so well shown. Either of the methods is fairly lengthy. The difficulty of direct accurate analysis of many organic materials also makes these methods troublesome for determining inside concentrations after elution of the solute from the resin phase for analysis.

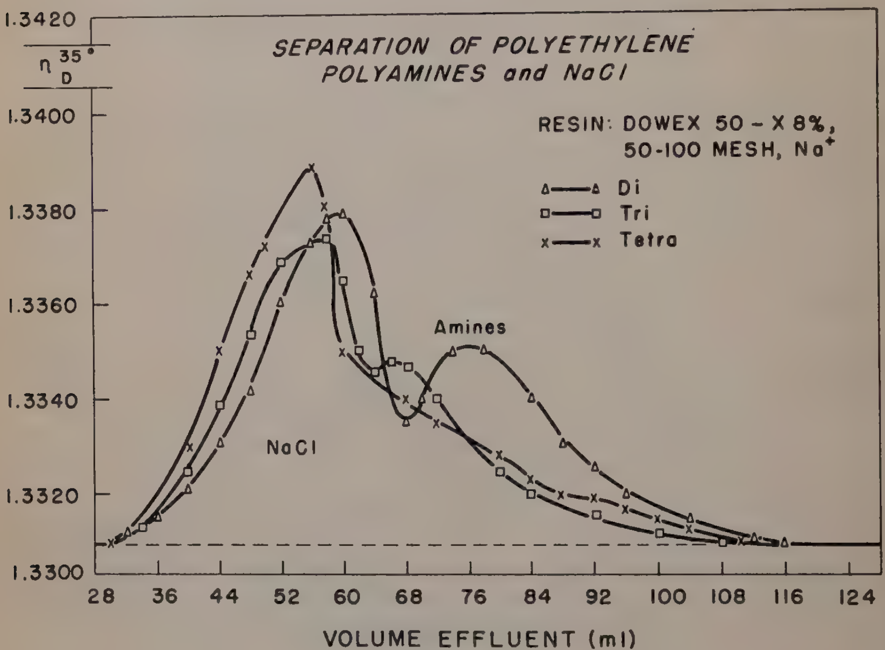

FIGURE 6.

Most of the laboratory ion exclusion type separations require 2–4 hours and a great many analyses. What is needed then is some quick method for determining the distribution constants short of a complete run. A procedure whereby K_d may be determined approximately but rapidly is as follows. A known quantity of resin of known water content is contacted with a known quantity of solution of known concentration. After equilibration the new outside solution concentration is determined and from this and resin volume changes the new inside concentration may be calculated. The concentration factors are considered on a weight/volume basis assuming that there is no intermolecular reaction between solute and solvent to change the total solution volume.

From these determined values K_d may then be calculated as follows:

$$K_d = \frac{C_{Si}}{C_{So}} = \frac{W_{Si}}{V_{Si} + V_{Wi}} \div \frac{W_{So}}{V_{So} + V_{Wo}}$$

$$V_{Si} + V_{Wi} = V_i$$

$$V_{So} + V_{Wo} = V_o$$

$$C^o_{So}V^o_o - C_{So}V_o = C_{Si}V_i - C^o_{Si}V^o_i$$

where C^0 and V^0 refer to initial conditions $C^o_{Si}V^o_i = 0$ by conditions of experiment.

All terms but C_{Si} are known.

$$C_{Si} = \frac{C^o_{So}V^o_o - C_{So}V_o}{V_i}$$

$$\frac{C_{Si}}{C_{So}} = \frac{1}{V_i}\left(\frac{C^o_{So}V^o_o}{C_{So}} - V_o\right)$$

$$= \frac{V_o}{V_i}\left(\frac{C^o_{So}V^o_o}{C_{So}V_o} - 1\right) = K_d$$

In the special case where there is no volume change, $V^o_o = V_o$, and

$$K_d = \frac{C_{Si}}{C_{So}}\frac{V_o}{V_i}\left(\frac{C^o_{So}}{C_{So}} - 1\right)$$

Concentrations may be expressed in any similar units, thus by refractive index (RI)

$$K_d = \frac{V_o}{V_i}\frac{(RI^o_o - RI_W)V^o_o}{(RI_o - RI_W)V_o} - 1$$

In general for non-ionic components there is very little resin volume change except at quite high concentrations and V_i approximates V^o_i. With ionic materials, such volume changes are quite appreciable.

The time required to reach equilibrium is dependent on the crosslinkage and particle size of the exchange resin and on the nature of the solute. Equilibration time increases as the surface/volume relationship decreases. This may be seen by a comparison of the times required to reach equilibrium using the 8 per cent crosslinked Dowex 50 at 20–50 mesh, 50–100 mesh and 200–400 mesh with 5 per cent ethylene glycol. The comparison is shown in FIGURE 7. Similarly for a given size resin, equilibrium is more rapidly attained with the lower crosslinked resins which are more loosely knit, more highly swollen (see FIGURE 8). Though these data are obtained with ethylene glycol it is un-

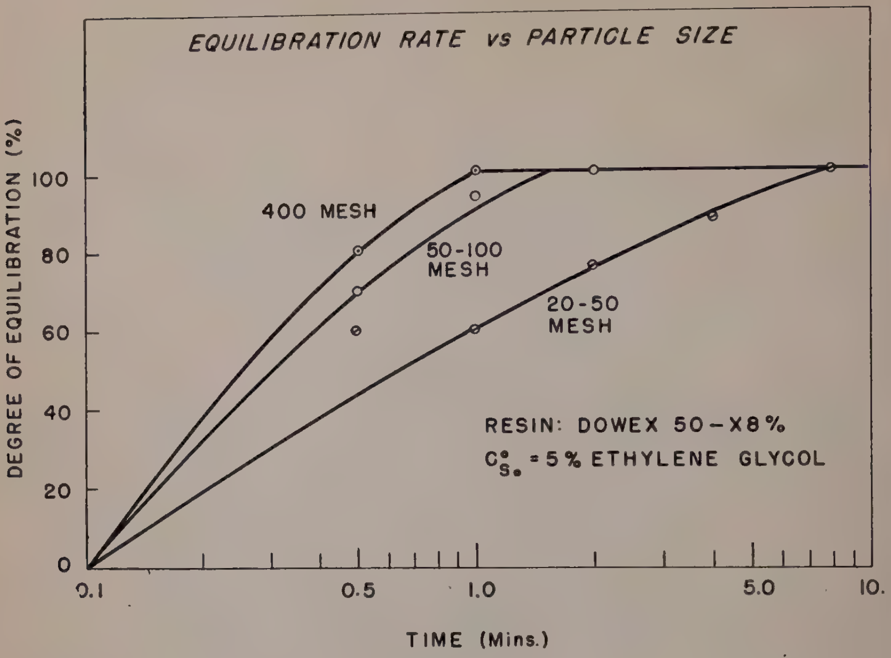

FIGURE 7.

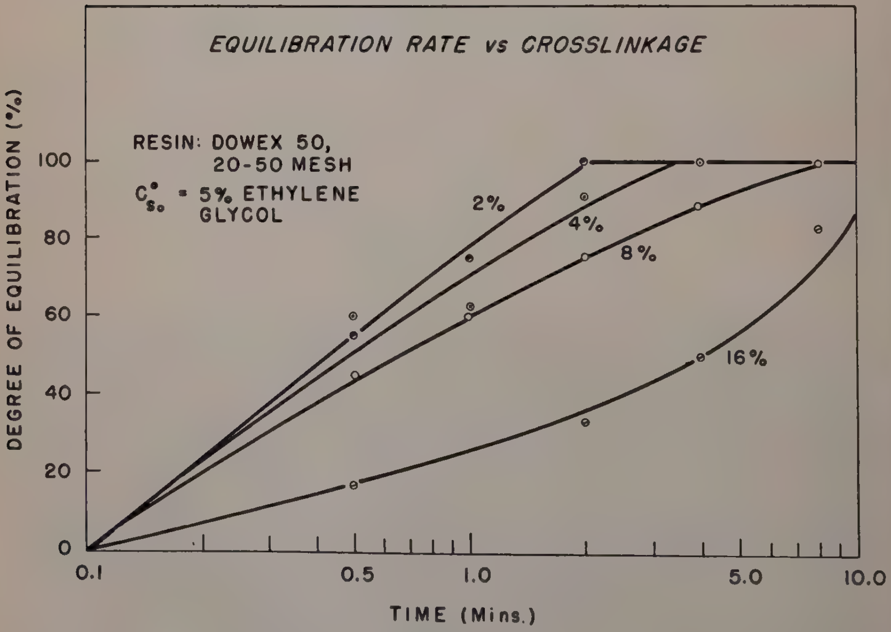

FIGURE 8.

doubtedly true that the same relationships hold true with any solute. The order of magnitude, however, may differ appreciably. For example, whereas a 5 per cent ethylene glycol solution requires 8 minutes for equilibration with Dowex 50–X8, H^+, 20–50 mesh, a 5 per cent triethylene tetramine solution requires 36 minutes because of its considerably lower diffusion rates.

As Gregor *et al.* have shown, the distribution of solute between the resin and outside solution phases is very dependent on a number of factors, including type of resin, ionic form of resin and temperature.

TABLE 1
MISCELLANEOUS DISTRIBUTION CONSTANTS
$C^o_{go} = 0.05$

Solute	Resin	K_d
Ethylene glycol	Dowex 50-X8, H^+	.67
Sucrose	Dowex 50-X8, H^+	.24
d-Glucose	Dowex 50-X8, H^+	.22
Glycerine	Dowex 50-X8, H^+	.49
Triethylene Glycol	Dowex 50-X8, H^+	.74
Phenol	Dowex 50-X8, H^+	3.08
Acetic Acid	Dowex 50-X8, H^+	.71
Acetone	Dowex 50-X8, H^+	1.20
Formaldehyde	Dowex 50-X8, H^+	.59
Methanol	Dowex 50-X8, H^+	.61
Formaldehyde	Dowex 1-X7.5, Cl^-	1.06
Acetone	Dowex 1-X7.5, Cl^-	1.08
Glycerine	Dowex 1-X7.5, Cl^-	1.12
Methanol	Dowex 1-X7.5, Cl^-	0.61
Phenol	Dowex 1-X7.5, Cl^-	17.7
Formaldehyde	Dowex 1-X8, $SO_4^=$, 50–100	1.02
Acetone	Dowex 1-X8, $SO_4^=$, 50–100	.66
Xylose	Dowex 50-X8, Na^+	0.45
Glycerine	Dowex 50-X8, Na^+	0.56
Pentaerythitol	Dowex 50-X8, Na^+	.39
Ethylene Glycol	Dowex 50-X8, Na^+	.63
Diethylene Glycol	Dowex 50-X8, Na^+	.67
Triethylene Glycol	Dowex 50-X8, Na^+	.61
Ethylene diamine	Dowex 50-X8, Na^+	.57
Diethylene triamine	Dowex 50-X8, Na^+	.57
Triethylene tetramine	Dowex 50-X8, Na^+	.64
Tetraethylene pentamine	Dowex 50-X8, Na^+	.66

TABLE 1 below gives random K_d values showing the variations with different materials and especially pointing out the variations resulting from changes of resin type and even of the ionic form of the resin. From these data one would predict that formaldehyde and acetone, for example, could be separated by either Dowex 50, hydrogen form or Dowex 1, sulfate form but with opposite order of elution. This prediction has been verified as shown in FIGURES 9 and 10. With Dowex 1, chloride form, there should be little separation. This also has been shown in laboratory experiments.

For a given solute and resin, it has been suspected that the distribution constant, K_d, would be variable with concentration. The general effect of change in concentration has not yet been determined. TABLE 2, however, shows the variation of K_d with concentration for ethylene glycol using Dowex 50-X8 hydrogen form. The steps in the calculations are indicated. The volume

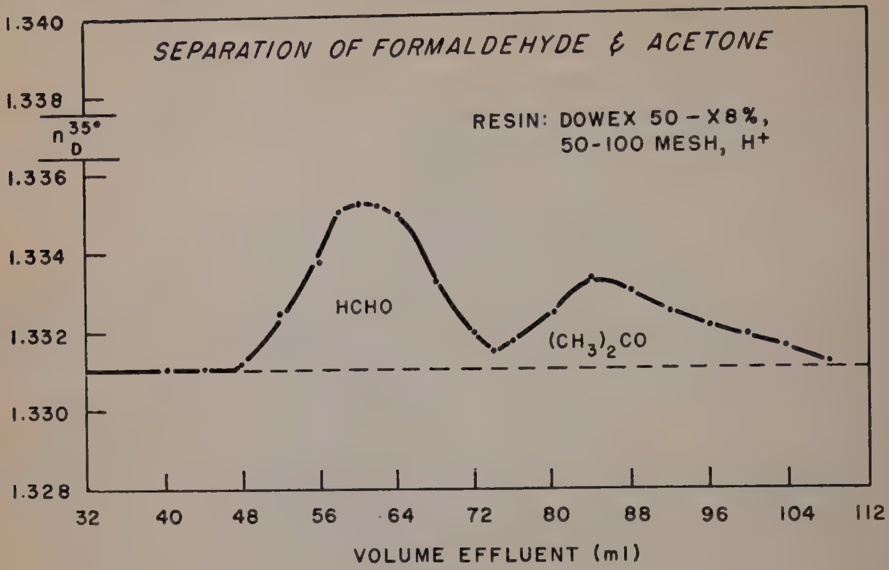

FIGURE 9.

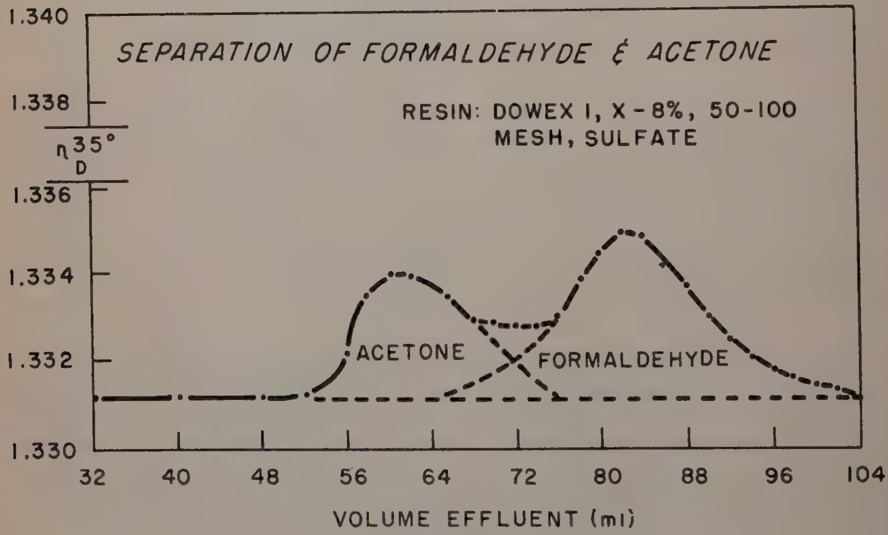

FIGURE 10.

change of Dowex 50-X8, H^+ in various ethylene glycol solutions has been independently determined and found to be *nil* in going from water to 50 per cent concentration. The total change in going from water to 100 per cent ethylene glycol is only −2.3 per cent. Some organic compounds are known to deswell the resin far more than this. In this series K_d varies only from 0.64 to 0.87 in going from 5 to 50 per cent ethylene glycol. In the earlier work it was shown that the location of acetic acid elution curves on separation from hydrochloric acid was almost identical regardless of its initial concentration. They differed only in height and thus average composition. From this it may be assumed that K_d for acetic acid also varies little with concentration.

Non-Ionic Elution

In the initial ion exclusion paper it was shown that the peaks of the effluent curve were related to the volume of water in the resin and that surrounding the resin (V_i and V_o). Assuming the ionic fraction to be completely excluded it should appear after V_o volume of solution has passed into the column following introduction of the feed. The non-ionic fraction on the other hand which ideally shows no preference between inside and outside solution phases should appear after $V_i + V_o$ volumes of solution had entered the column. For hydrochloric acid and acetic acid and a great many other systems this is closely approximated. However, with known K_d values the location of the leading edge of an effluent curve may be more accurately predicted. Thus:

TABLE 2

DISTRIBUTION CONSTANTS FOR ETHYLENE GLYCOL WITH DOWEX 50-X8·H⁺

$C^o_{S_o}$	$V^o_{S_o}$	$V^o_{W_o}$	V^o_o	V^o_i	Start n^{25}_D	Final n^{25}_D	C_{S_o}	V_o	V_i	K_d
.050	2.33	50.0	52.33	50.0	1.3360	1.3342	.0310	52.33	50.0	0.64
.101	4.91	50.0	54.91	50.0	1.3409	1.3370	.0598	54.91	50.0	0.76
.207	11.2	50.0	61.2	50.0	1.3505	1.3430	.126	61.2	50.0	0.78
.312	19.1	50.0	69.1	50.0	1.3600	1.3495	.195	69.1	50.0	0.83
.530	44.3	50.0	94.3	50.0	1.3798	1.3650	.363	94.3	50.0	0.87
.530	29.4	33.25	62.65	50.0	1.3803	1.3612	.320	62.65	50.0	0.83

$$V_{max.} = V_o + K_d V_i$$

where $V_{max.}$ = volume of effluent eluting from column from time of introduction of feed to the peak concentration of an infinitely small sample of effluent of the given K_d. That this is not exactly the case is explained by the fact that K_d is not a true constant (above). Also, it appears from analysis of elution curves that even where there is no suspected chemical reaction between solutes the distribution constants are affected. Thus, it has been noted several times that two solutes separate better in practice than theory would predict. This is consistent with the findings of investigators of more conventional chromatography.

The general correlation between the calculated $V_{max.}$ and the elution curves of single components is shown in TABLE 3. The curves are analyzed at the leading edge at a point midway in concentration between water and the peak concentration. S refers to elution of single components, M of multiple components.

The choice of resins which may be used for separations of non-ionic components is much wider than where an ionic component is involved. If an ionic component, *e.g.*, sodium chloride, is present the resin choice is limited to a cation or anion exchanger having an ion common to the salt, sodium, or chloride. If not, the resin will be converted to that form. The only prerequisite for the non-ionic materials is that they be chemically unreactive with the resin. For example, an amine which is very weakly ionized may not be fractionated on the hydrogen form of a strongly acidic cation exchanger. Up to the present we have been unable to develop a theory by which we can choose the proper resin except by trial and error using the distribution constants or trial runs. We have noted that separations are often more complete with the hydrogen form of Dowex 50 than with salt forms. This may reflect the fact that the acid form is more organic in nature. Linear polystyrene sulfonic acid is soluble in some organic solvents, *e.g.*, methanol, while the salt forms are not.

Table 3

Comparison of V_{max} with Elution Curve Analysis

Solute	Resin	Elution*	K_d	V_{max}	Leading Edge	Error (%)
Sucrose	Dowex 50-X8, H$^+$	S	0.24	40	39	−2.5
Glycerine	Dowex 50-X8, H$^+$	S	0.49	51	57	+11.8
Triethylene Glycol	Dowex 50-X8, H$^+$	S	0.74	60	67	+11.6
Acetic Acid	Dowex 50-X8, H$^+$	S	0.71	61	60	−1.7
Ethylene Glycol	Dowex 50-X8, H$^+$	S	0.67	58	62	+6.9
Sucrose	Dowex 50-X8, H$^+$	M	0.24	40	40	±0.0
Glycerine	Dowex 50-X8, H$^+$	M	0.49	51	60	+17.7
Triethylene Glycol	Dowex 50-X8, H$^+$	M	0.74	61	70	+14.8
Phenol	Dowex 50-X8, H$^+$	M	3.08	159	178	+12.0
Acetic Acid	Dowex 50-X8, H$^+$	M	.71	60	61	+1.6
Acetone	Dowex 50-X8, H$^+$	M	1.20	80	78	−2.5

* S = Single Component Feed. M = Multiple Component Feed.

As with ion exclusion, separations may be improved with reduction of particle size, because of more rapid equilibration (see above). It is thus seen that to get comparable separations flow rates must be decreased as mesh size increases. Practically, this has its limitation in the fact that back diffusion becomes more pronounced as particle size increases, actually causing very broad elution curves even with extremely slow flow rates with coarse resins, *e.g.*, 20–50 mesh. Conversely, better elution curves with the fine resins suggest the use of the finest resins available. Flow rate now becomes a problem, not because of equilibrium but, because of the pressure drop through the column. It should be pointed out here that pressurizing up to several atmospheres is not a serious cost factor in most operations and that the commercial use of resins much finer than now considered practical is advocated.

Though the elution curve is sharper for a given component with a lower crosslinked resin, the point is reached where the separation factors diminish and K_d approaches 1 which would be the case for a completely soluble resin of no crosslinkage. Studies have been made with ethylene glycol and various crosslinked resins. The K_d values for the 16, 8, and 1 per cent crosslinked Dowex 50 resins were 0.33, 0.62, and 0.88 respectively indicating this approach to $K_d = 1$ with lower crosslinkage. These were determined with 5 per cent ethylene glycol solutions.

As stated earlier, the elution curve for a given component through an ion exchange resin is not greatly affected by the presence of other components unless there is some interaction between the two components or the resin volume changes appreciably because of one of the components. Thus, by observing elution curves for single components it is generally possible to predict the degree of separation possible with a mixture of the components. This is evident in FIGURE 11 which shows three separate runs on the same column in each case duplicating operating conditions as closely as possible. Separate runs were made with d-glucose and acetone solutions (as shown by the curves) followed by a run using a mixture of the materials each at the same concentration as in the first runs. From the first two curves, the summation curve may be calculated (squares) and this is compared with the actual experimental run (crosses).

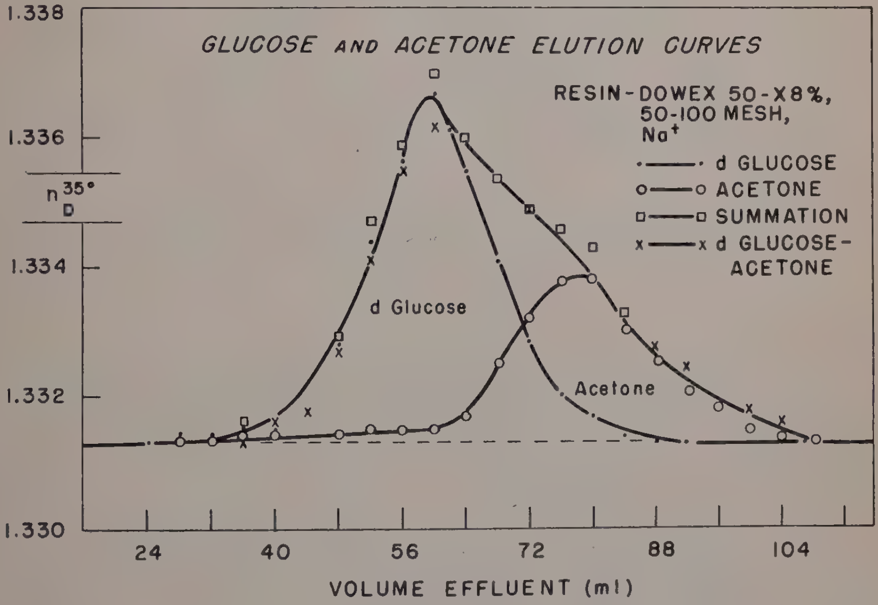

FIGURE 11.

Similarly, the elution curve of a component in a given experiment may be used to determine its shape and location in another separation. For example, both acetic acid and acetone can be separated from d-glucose using the hydrogen form of Dowex 50. Acetone is, however, much more completely separated than is acetic acid. In the effluent curve from the 100 ml. column the acetic acid peak concentration appears at about 67 ml. and acetone at 86 ml.

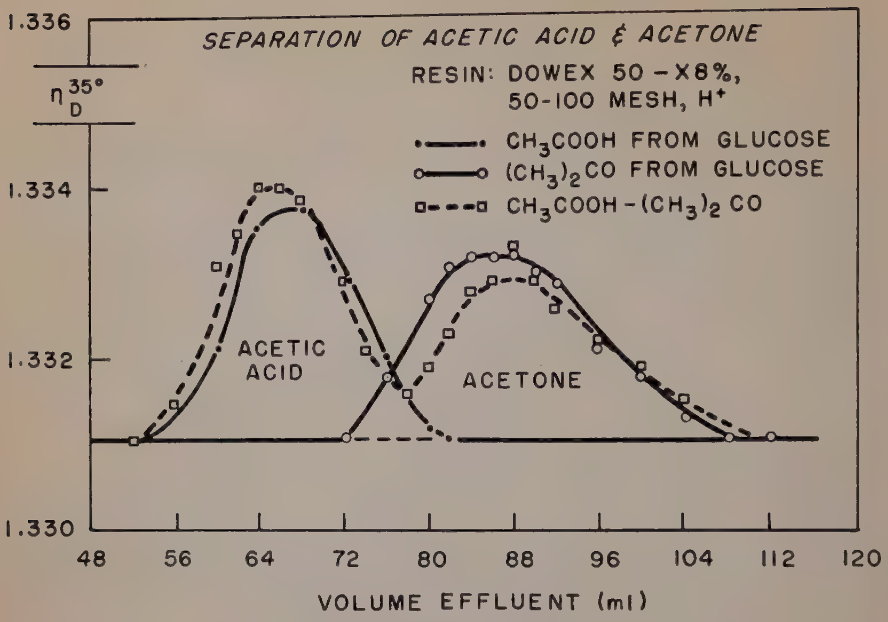

FIGURE 12.

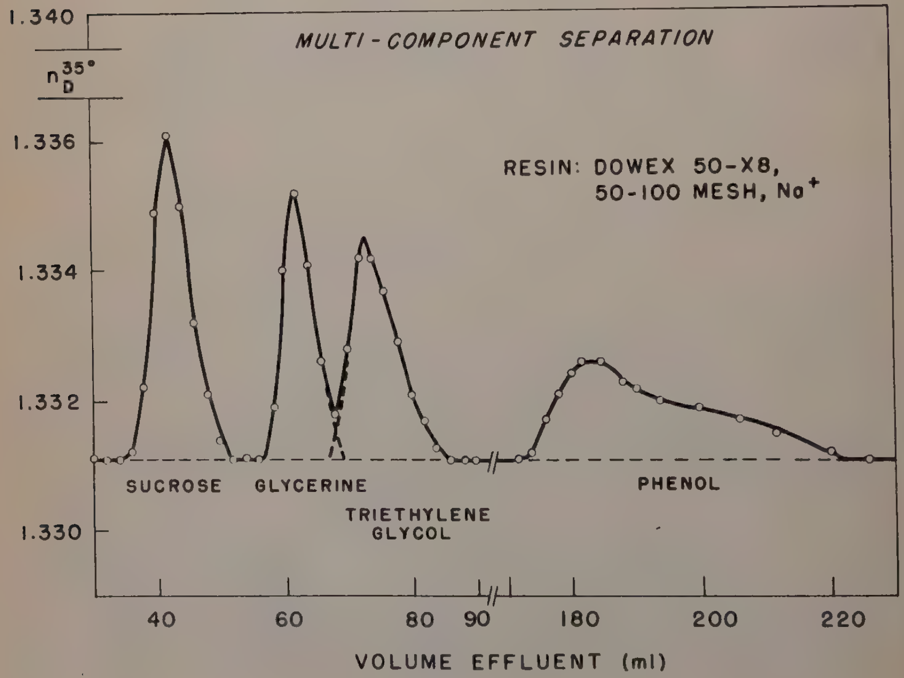

FIGURE 13.

From this, it would be expected that the two components could be readily separated using the same resin. This was accomplished very satisfactorily with peaks at 65 and 88 ml. respectively, which very closely approximates the expected elution curves. See FIGURE 12.

Up to this point, we have concerned ourselves strictly with two-component separations. With the knowledge, however, that a wide spectrum of K_d values exist for different compounds on a given exchanger, there is every reason to believe that separation of a considerably larger number of components is very possible, and it should be possible to predict with fair accuracy the location of each of the components. In FIGURE 13 is shown a four-component separation on Dowex 50–X8, 50–100 mesh. The feed consisted of 5 ml. of solution containing 5 per cent each of sucrose (K_d = 0.24), glycerine (K_d = 0.49), triethylene glycol (K_d = 0.74) and phenol (K_d = 3.08). There is seen to be some overlapping of glycerine and triethylene glycol under the conditions of the run. Phenol which is so much more effectively sorbed by the resin is separated from the triethylene glycol by about 90 ml. of water. Phenol is even more strongly held by the strongly basic anion exchange resins, *e.g.*, with Dowex 1–X 7.5, chloride form, K_d = 17.7.

In general terms, any differential in K_d values means that a separation of the components in question is possible if feed, resin, and operating conditions are properly controlled.

Applications

Whereas the separation of non-ionic components by ion exclusion methods does not show as much promise for immediate commercial applications as does ion exclusion itself, it does add a wide degree of utility to ion exchange resins. It is very likely to have immediate application in the analytical field. For example, two compounds of similar chemical properties may be present in mixture, each interfering with the analysis of the other. If they can first be separated and identified, quantitative analysis is much simplified. One such example has been encountered in the case of a chloroacetaldehyde fraction which was believed to contain dichloroethanol as an impurity. An attempted separation as described herein yielded two fractions and the dichloroethanol could be positively identified. Subsequently, the dichloroethanol could be determined with fair degree of accuracy by simply measuring the area under its curve and comparing with a known value.

Compounds which are very strongly sorbed by the resin, such as phenol and the substituted derivatives of phenol are on strongly basic anion exchange resins, may be separated from other non-ionics or ionic compounds. This may be used as a method for purifying the phenolics or conversely, a method for removing the phenols from waste solutions.

Summary

Ion exchange chromatography in its purest form is very possible without the use of any regenerant or displacement chemicals, other than water. Simple elution of compounds having different inside-outside distribution constants with water, or other polar solvent, can in many cases give very good separations. The method is of particular value for fractionation of compounds not otherwise easily separated.

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ION EXCHANGE MEMBRANES

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Introduction

The importance of membranes in physical chemistry as well as in the biological sciences is due to their twofold character. On the one hand, membranes are restrictive barriers which selectively influence the transportation and exchange by diffusion, osmosis or otherwise of the various molecular and ionic species contained in the two compartments which are separated by the membrane. On the other hand, membranes, by virtue of their being barriers, act also as physicochemical machines which regulate the flow of the energetic processes which occur across their thickness; and, in doing this, they transform various forms of energy into others, for instance, osmotic energy into mechanical work or into electrical energy.[1]

Membranes which are known today to be of ion exchange character have been investigated for many decades by physical chemists, and more extensively by biologists. Virtually all the fundamental electrochemical membrane phenomena were thoroughly described and basically understood before the true underlying physical mechanism, the ion exchange nature of the membranes, was clearly realized. After the ion exchange character of electrochemically active membranes was recognized and extensive theoretical and experimental work started on this new basis, it was more than ten years before membranes prepared from conventional, commercial type ion exchanger material were described. Thus, ion exchangers of the high capacity, commercial type which, after all, are only one special class of a much larger group, are late comers in the membrane field. Up to the present, hardly a single significant, new observation has been made with a membrane prepared from the commercial type of ion exchangers which has not been described earlier and with a higher degree of accuracy.

At present, numerous investigators here and abroad, using commercial type ion exchangers, are beginning to study the electrochemistry of membranes, primarily looking for technological applications. There is little doubt in the mind of the author that, in the long run, membranes, prepared with the aid of methods more or less common in ion exchange technology, will assume industrial importance.[2]

The current process of merging of the knowledge of electrochemistry of membranes with the vast body of experience in the ion exchanger field is bound to be of great mutual benefit. It is too early at present to predict where the first "big break" from an industrial point of view will come, and in which direction the membrane technology of the future will develop.

The main purpose of this paper is not to indulge in speculations along these lines, but rather to present the fundamental aspects of the electrochemistry of membranes of ion exchange character; to indicate the methods of preparing membranes of highest electrochemical activity including, as far as published, information concerning membranes prepared from the more conventional type of ion exchangers; and to illustrate by examples the usefulness of the best types of membranes in various types of physicochemical studies and laboratory procedures, particularly of biochemical and physiological interest. For obvious reasons, work from the author's laboratory will be stressed in this report.

The Fundamental Electrochemistry of Porous Membranes of Ion Exchange Character

In a discussion of the physical chemistry of membranes it is convenient to distinguish two main classes of membranes, "homogeneous phase membranes" (oil membranes) and "membranes of porous character." Homogeneous phase membranes exert their typical membrane functions by means of selective, differential solubility. Particles, molecules, or ions which are soluble in an oil phase can penetrate and pass across it. Membranes of porous character act as sieves which screen out the various species of solute particles according to their different size, to some extent their different adsorbabilities and, in the case of ions, also according to the sign and magnitude of their charge. The membranes of ion exchange character to be discussed here fall into this latter class: they are membranes of porous character.[1, 2]

Membranes of porous character can be arbitrarily classified into two groups: membranes of high porosity, as exemplified by ordinary dialyzing membranes; and "molecular sieve" or "ion sieve" membranes with pores so narrow that different low-molecular weight species of molecules and ions are retarded to a differential degree, or prevented altogether, from passing across them. Membranes of both types of porosity are found in living organisms and are therefore of primary biological importance.

Of particular interest is the interplay between such membranes and solutions of electrolytes; that is, the electrolyte or, more correctly, the ion permeability of the membranes; and the concomitant electrical phenomenon: the functional electrochemistry of membranes.

The basic observations in the electrochemistry of membranes refer to their electromotive action which becomes conspicuous when a membrane separates two solutions which are not identical with respect to their electrolyte content, the simplest case of this nature being the membrane concentration chain.

If a membrane prepared from collodion, silicates, proteins, or almost any other material is interposed, *e.g.*, between two solutions of different concentration of the same electrolyte, an electromotive force arises, that is different in most instances from the liquid junction potential which would arise between the same two solutions on free diffusion, that is, in the absence of a membrane. The electromotive forces arising in such membrane concentration chains customarily are referred to as "concentration potentials."

The sign and the magnitude of the concentration potential depends on the absolute concentrations and the concentration ratio of the electrolyte in the two adjacent solutions, also on its nature and last, but not least, on the nature of the membrane.

With membranes of highest porosity (porous diaphragm) the concentration potentials are in sign and magnitude identical with or not much different from the corresponding liquid junction potentials. The concentration potentials deviate more and more from the liquid junction potential if stepwise denser membranes are used.

The direction of the deviation of the concentration potential from the liquid junction potential is functionally correlated with the electrokinetic charge of the membrane. With electropositive membranes, the dilute solution is more negative; with electronegative membranes, more positive than on free diffusion. From this it may be concluded that electronegative membranes are preferentially cation permeable, electropositive membranes preferentially anion permeable, as is readily confirmed by direct observation. This is the most fundamental fact in the whole electrochemistry of membranes of porous character.

With certain membranes of very low porosity, the concentration potential may reach the magnitude of the potential difference which would arise between the two solutions, if they were connected to each other through a pair of reversible electrodes, specific either for the cations or the anions in solution, as the case may be. This "thermodynamically possible maximum value" of the concentration potential represents the upper limit of the possible membrane concentration potentials, the liquid junction potential being the other limit.

The correlation of ionic membrane selectivity and concentration potential in formal electrochemical terms is the basic concept in the electrochemistry of these membranes. It can be readily visualized along the following lines of thought. The virtual transportation of electricity across the membranes is divided between anions and cations in a proportion which is different from the ratio of the *transference numbers* of these ions in free solution.[3] In electronegative membranes, a greater fraction of the current is transported across the membrane by cations than in free solution, and the transference number of the cations in the pores of the membrane (τ_+) is larger than the transference numbers of the cations in free solution t_+ ($\tau_+ > t_+$; and $\tau_- < t_-$). With positive membranes the inverse holds true.

The correlation of membrane concentration potential, ϵ, and the transference numbers, τ_+ and τ_-, may be expressed quantitatively by the use of a modified Nernst equation. For the case of a uni-univalent electrolyte and a negative membrane, it reads:

$$\epsilon = \frac{\tau_+ - \tau_-}{\tau_+ + \tau_-} \frac{RT}{F} \ln \frac{a_\pm^{(2)}}{a_\pm^{(1)}} \tag{1}$$

where $a_\pm^{(1)}$ and $a_\pm^{(2)}$ are the activities of the electrolyte in the two solutions, the sum of τ_+ plus τ_- by definition being unity.

If a membrane is exclusively permeable to cations, the transference number of the cation in the membrane, τ_+, is unity. It is an electronegative "*membrane of ideal ionic selectivity.*" Correspondingly, an electropositive membrane of ideal ionic selectivity is permeable exclusively to anions, τ_- being unity.

The essence of the physical situation which explains the correlation of the electrokinetic charge of membranes and their electromotive action has been clearly understood for a long time. The charges (ions) which form the immovable part of the electric double layer at the pore wall/solution interface are attached firmly to the pore walls. They are unable to move and thus do not participate in the transportation of electricity. The counter ions of the fixed wall charges are dissociated off into the liquid in the pores, freely movable and therefore able to participate in the transportation of electricity, the current being transported across the membrane by these ions and whatever other electrolyte, both anion and cation, may be present in the pores. Thus, the movable counter ions of the fixed wall charges are the vehicle for a larger and larger fraction of the virtual or actual transportation of electricity in the membranes as membranes of decreasing porosity are considered.

Ions of the same sign of charge as the membrane are prevented by electric repulsion from approaching the spots at the pore walls, where the fixed charges of the same sign are located. For sufficiently narrow pores such ions (and therefore their electrically compensating counter ions, too) are virtually excluded. In this case, the membrane acts as a membrane of ideal ionic selectivity; and all the possible pathways across it are blocked completely for the ions in solution which carry the same charge as the membrane itself.

With increasing concentration of the outside electrolyte solutions, an increasing quantity of electrolyte, equivalent quantities of anions and cations, enter the pores. The specific influence of the membrane is thereby decreased. This explains why the ionic selectivity of a given membrane decreases if the concentration of the adjacent electrolyte solutions is increased.

Polyvalent ions with a charge of the same sign as the membrane are much more restricted in their permeation across the membrane than univalent ions, because of their large size and because of their high charge, that prevents them by electric repulsion from entering narrow pores which are accessible to univalent ions of the same sign.

At this point, it is necessary to introduce the often neglected but highly important concept of membrane heteroporosity.[4] Any membrane which is available at present for experimental investigation must be assumed to be heteroporous, a mosaic of wider and narrower channels.[5, 6] The pores are the interstices between micelles which are arranged in a more or less random manner. The observable membrane effects are the gross result of the processes which occur across the different pores and arise due to their interaction. Certain of the consequences of heteroporosity were stressed by Collander,[7] Michaelis,[3] and Sollner.[4, 8, 9, 10]

For the foregoing considerations, it has not been necessary to make any special assumptions as to the specific mechanism by which the charge of the membranes arises. From the formal point of view, it could be considered to be immaterial up to a point, though it is of primary importance for the further conceptual development of the theory, and any attempt at a quantitative theoretical treatment.

Twenty years ago, the nearly universal opinion among colloid and surface chemists was that the charge of the common, inert materials from which membranes were customarily prepared such as collodion, is due to the adsorption of ions from solution. While this concept holds true in certain instances, it has been found to be incorrect as far as the interplay between solutions of strong inorganic electrolytes and any common membranes is concerned. The basic electrochemical behavior, even of the presumably inert materials, such as, for instance, collodion, is due to dissociable groups such as carboxyl groups (probably stray and end groups) which form an inherent, integral part of the molecules, or at least a fraction of the molecules, of these substances, and are not shown in the conventional formulas. Thus the supposedly inert materials, for instance, the commonly used collodion, are strictly speaking ionizable substances and therefore, in their electrochemically important properties, essentially identical with materials like clays, zeolites, or protein gels, which are customarily classified as *ion exchangers*. If acidic in character they are cation exchangers; if basic, anion exchangers. The difference between these substances and the more inert materials is only of a quantitative nature. Potentially dissociable groups are distributed much more sparsely on the surfaces of the "inert" materials than on the substances which are commonly referred to as ion exchangers, the latter having a much higher exchange capacity per unit of accessible surface area.[1, 11]

In many instances, membrane materials, nearly devoid of electrochemically active, dissociable groups, have been "activated" by the adsorption of high molecular weight, dissociable substances, such as proteins. The latter, like other polyelectrolytes, when adsorbed on an inert membrane skeleton, impress on its microstructure the essential property of ion exchangers, a definite and usually fairly high charge density or number of fixed dissociable groups per unit area.

The conception of membranes as ion exchange bodies leads to the presentation of the fixed charge theory of electrical membrane behavior. This theory, on the basis of a clear physical picture, namely the ion exchange character of the membranes, correlates in a quantitative manner the previously outlined facts and ideas concerning membrane selectivity and the concomitant electrical effects.

The physical essence of the fixed charge theory can be stated qualitatively in a simple manner. The rather elaborate mathematical terms in which this theory was originally presented by Teorell[12] and later in much greater detail by Meyer and Sievers[13, 14] are far too complex to be outlined here.

According to the fixed charge theory the walls of the pores of the membranes carry inherently a definite number of potentially dissociable groups, anionic (acidic) groups, such as carboxyl groups, in the case of electronegative membranes, and cationic (basic) groups, such as amino groups, in the case of electropositive membranes. These dissociable groups are an integral, invariable part of the membrane structure. Their number is independent of the nature or concentration of the adjacent electrolyte solutions. Any current which flows across the membrane is transported by the counter ions of the fixed charged wall groups, and whatever other "nonexchange" electrolyte may be present in the pores. The concentration of the latter is determined by a Donnan equilibrium existing between the electrolytic pore structure of the membrane and the adjacent outside electrolyte solution, the fixed wall groups being the "nondiffusible" ions. In a concentration chain, two such Donnan equilibria are set up between the two solutions and the two adjacent surface layers of the membrane, with a corresponding concentration drop across the membrane. The concentration potential is considered as the algebraic sum of the two Donnan potentials plus the liquid junction potential within the pores.

Although based on assumptions which are oversimplified as far as membranes of porous character are concerned, the core of the fixed charge theory is destined to play a dominant role in the further development of the theory. Whether it will be ultimately advisable to split up the observed membrane potential into three parts, two nonoperational distribution potentials (of doubtful meaning) and one diffusion potential, or whether it may not be preferable to consider statistically the probability that individual ions will pass critical spots in a heteroporous structure, cannot yet be decided. The former method of approach has the advantage of being based on rather fully developed concepts; but it will be applicable only after considerable modifications to the actual physical situation which involves microheterogeneous interphases, where the conventional electrochemical concepts of phase boundary potentials, for instance, become blurred. The latter method of approach, namely, a consideration of molecular processes occurring at individual spots, might be much closer to the physical facts and conceptually less involved. However, it could not be connected quite as conveniently to prior work in theoretical electrochemistry.[1, 4, 11]

Preparation and Properties of Membranes of High Electrochemical Activity

The fixed charge theory implicitly gives directions towards the preparation of membranes of highest electrochemical activity. It postulates that, *ceteris paribus*, the electrochemical properties of membranes of porous character will be the more pronounced the greater the number of potentially dissociable groups per unit area on the walls of the pores. This is of great importance because the experimental study of the fundamental electrochemistry of membranes and their use in model studies has been retarded in the past, to a great extent, by the lack of suitable objects for such investigations. Now, however, one can plan systematically for the preparation of membranes which are suitable—tailor made, so to speak—for particular purposes. The obvious objective was to prepare membranes which would yield the electrical and permeability phenomena under investigation to a very pronounced degree. In particular, with membranes of ion sieve character, it was obvious to strive for membranes which would be of virtually ideal ionic selectivity. At the same time, these membranes should allow a fast rate of diffusion of the critical ions across their thickness. In other words, they should be membranes of low resistance. Such membranes would be much more useful for physicochemical experimentation, and likewise in model studies of biological interest. For this type of ion sieve membranes of highest ionic selectivity and high permeability, the denotation "megapermselective" or "permselective" membranes was suggested, the latter term being now commonly used.[15]

The current state of the methods of preparation of membranes of highest electrochemical activity is discussed in the subsequent paragraphs, together with the description of the methods used for their characterization.

Membranes of high electrochemical activity, that is, membranes with relatively high charge density (or number of potentially dissociable groups) per unit area can be prepared by different methods. One may use a membrane-forming material which carries inherently an adequate number of dissociable groups (commercial type, high capacity ion exchangers are an extreme example in this direction); or an activating material of polyelectrolyte nature may be dissolved in the solution of an electrochemically fairly inert material, such as collodion, from which membranes are prepared. Electrochemically active polyelectrolyte molecules are thus embedded, entwined and enmeshed in the inert matrix when the membrane is formed. In so far as they are located in the accessible pore surfaces, they act there as typical ion exchangers; or a powder or granules of ion exchanger are cemented together to form a membrane. Another method, often used, consists of activating an inherently inert membrane by the adsorption of some polyelectrolyte, which attaches itself in a virtually irreversible manner to the preformed membrane structure. Finally, membranes prepared from inactive material may be activated by a chemical reaction, for instance, oxidation, which results in the formation of dissociable groups (carboxyl groups) on the accessible pore walls of the preformed membrane structure.

These several general methods of preparing membranes of highest electrochemical activity lend themselves with different ease to the preparation of membranes of varying porosity. In particular, up to now, seemingly no attempt has been made to prepare membranes of high porosity by the use of commercial type ion exchangers or the general methods of organic synthesis used in this latter field.

Electronegative membranes of high porosity and a degree of electrochemical activity which previously had never been obtained have been prepared by the use of collodion which had been oxidized in bulk;[16] by the dissolution of polyelectrolytes such as polyacrylic acid or sulfonated polystyrene in the collodion solution from which the membranes are prepared;[17, 18] by the adsorption method;[17, 18, 19] and, by chemical reaction, the oxidation of formed collodion membranes.[16, 20, 21] The latter method, for our purposes, has the advantage that it permits the comparison of the same membrane in a relatively inactive nonoxidized state and, in the oxidized state after numerous, additional dissociable groups have been formed throughout its microstructure. The membranes referred to in the subsequent paragraphs are of this latter type, their functional electrochemical properties being virtually identical with those of membranes prepared by the other methods.

The two basic electrochemical parameters for the characterization of membranes of high porosity are the membrane concentration potential and the rate of electro-osmosis across them, the latter being determined by the electrokinetic potential of the membranes. The higher the concentration potential and the higher the rate of electro-osmosis, the greater the "electrochemical activity" of a membrane of given porosity.

The influence of oxidation on the concentration potential across a formed membrane is seen from these data. A membrane of the dialyzing type, before oxidation, gave in the concentration chain KCl 0.1 *N*/membrane/KCl 0.01 *N* a concentration potential of 1.6 millivolts, after oxidation 25.0 millivolts.

The behavior of the membrane towards nonelectrolytes, as must be stressed, was not significantly changed by the oxidation.

The influence of oxidation on electro-osmosis can be seen in columns 1–3 of the self-explanatory TABLE 1. The influence of changing the ion exchange characteristics of the pore walls of the membrane is apparent from these data.

Another rather sensitive and very convenient way to define empirically the electrochemical activity of membranes of high porosity is the study of the rate of anomalous osmosis across them. This phenomenon, well known and much investigated by biologists, according to the experiments of Girard,[22] Bartell,[23] Loeb,[24] and others is essentially a spontaneous electro-osmosis which occurs when an electrolyte diffuses across a charged membrane. Its physical theory had been developed several years ago by the author who based his considerations on the heteroporosity of the membranes.[8, 9, 10] The driving force of anomalous osmosis is due to the electrolyte diffusion itself, being functionally related to the dynamic membrane potential. Thus the rate of anomalous osmosis is dependent, both on the magnitude of the electrokinetic potential of the membrane and, in a rather complicated manner, on the dynamic membrane potential. It is, therefore, a particularly sensitive indicator of the electrochemical activity of membranes of the dialyzing type. It has been used extensively in recent years for the characterization of such membranes,[1, 4, 16, 20, 21, etc.] particularly in view of the great interest which biologists have shown in this phenomenon for many decades.

TABLE 1

ELECTRO-OSMOSIS THROUGH A TYPICAL UNOXIDIZED COLLODION MEMBRANE OF HIGH POROSITY, THE SAME MEMBRANE AFTER OXIDATION, AND A PROTAMINE COLLODION MEMBRANE OF ABOUT EQUAL POROSITY

1	2	3	4
Concentration of KCl Solution	Electro-osmotic flow in mm^3 per 100 cm^2 per hour with a current intensity of 0.1 milliamp per cm^2*		
	Unoxidized collodion membrane	Oxodized collodion membrane	Protamine collodion membrane
equivalents/liter	*mm^3*	*mm^3*	*mm^3*
0.00100	+2840	+4640	−4380
0.0100	+360	+1800	−1680
0.100	±0	+210	−80

* A plus sign indicates transport towards the cathode, a minus sign transport towards the anode.

Space does not permit reviewing here the details of Loeb's well known experimental method of studying the transitory, dynamic phenomenon of anomalous osmosis.[1, 20, 21, 24, etc.] Suffice it to say that membrane bags fitted with capillary glass tubes are filled with solutions of various concentrations of different electrolytes and suspended in a beaker filled with distilled water. The pressure rises observed after a standard time, usually 20 minutes, are noted. The difference between the rise with electrolytes and a nonelectrolyte reference substance is commonly considered as a measure of anomalous osmosis.

FIGURE 1 allows the comparison of the activity of a membrane of Loeb's which was prepared from an impure collodion preparation which contained a significant quantity of dissociable groups (FIGURE 1A);[1, 16, 20] of a membrane of about the same porosity prepared from pure commercial collodion which is nearly devoid of dissociable groups (FIGURE 1B); and of the same membrane after oxidation whereby a considerable number of dissociable groups was created at the walls of its pores (FIGURE 1C). The abscissae give the concentration of the solution inside the test tube-shaped membrane immersed in distilled water. The ordinates represent the pressure rise (in mm water) observed after 20 minutes. As can be seen from the FIGURES 1B and 1C, the behavior of the membranes with solutions of nonelectrolytes is hardly changed by oxidation.

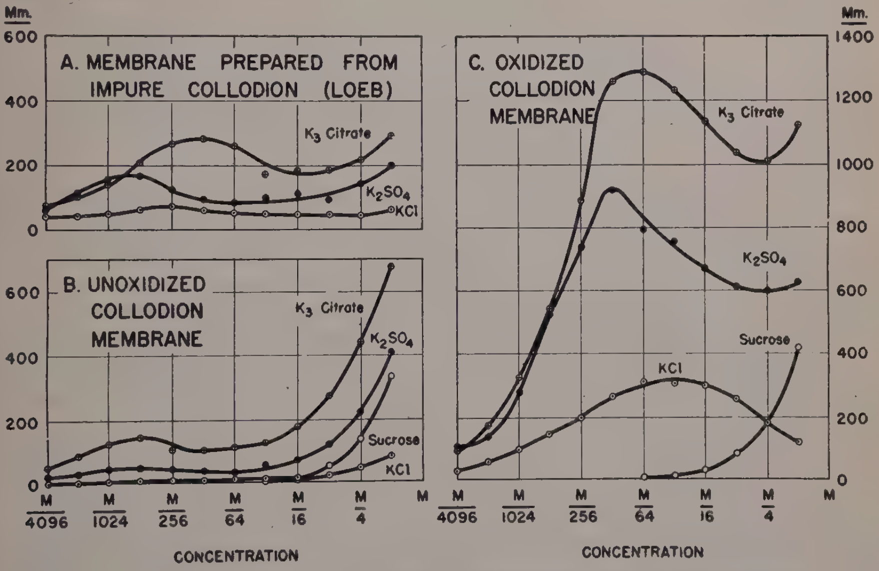

FIGURE 1. A comparison of the extent of anomalous osmosis through various collodion membranes of about equal porosity.

It might be added that oxidation beyond a certain point does not increase the functional electrochemical activity of these membranes as studied with the outlined methods.

The exchange capacity of even the most highly active oxidized collodion membranes is extremely low, too low to be measurable with accuracy.[11, 25] The inherent acidity of even rather active preparations is exceedingly small, the equivalent weight of highly active preparations being of the order of 30,000 as compared with 184 for a (mono-) sulfonated polystyrene type of commercial ion exchanger. Of these sparsely present active groups in collodion, only a small fraction is functionally available for ion exchange or membrane effects![11, 26]

Highly active electropositive (basic) *membranes of high porosity* have been obtained by the adsorption, on collodion membranes, of protamines, basic proteins with an isoelectric point of about pH 12 and a molecular weight of around 3000.[27] These membranes are extremely stable and maintain their essential characteristics over a wide pH range, about 2.8 to 8.5. Highly porous protamine collodion membranes with respect to concentration potential, electro-osmosis (see column 4 of TABLE 1) and anomalous osmosis are the exact analogues to the corresponding electronegative membranes described in the preceding paragraphs.[1, 27]

Parenthetically, it might be remarked here that anomalous osmosis across membranes of high porosity has intrigued biological investigators for a long time as a possible mechanism to explain translocation of liquids in the living organism, particularly the mammalian organism. All the results on anomalous osmosis, however, which were obtained by the method of Loeb are of doubtful significance for the latter purpose, since they were obtained with systems in which distilled water was on the one side of the membrane, a situation which is far from that prevailing in the mammalian organism. Several prominent investigators, such as Loeb,[28] Freundlich,[29] Höber,[30] and recently Teorell[31] have doubted that anomalous osmosis can occur at all at concentrations above 0.05 N since the phenomenon is of electrokinetic nature. We, therefore, have carried out recently some studies[32] in which membranes separated different electrolyte solutions from each other, both solutions being at the physiological concentration level. The main result of these studies is that, with electronegative as well as with electropositive membranes of high electrochemical activity and with properly chosen solutions, anomalous osmotic effects can occur which might amount to 30 ml. per 100 cm.^{2} of membrane per hour. These rates are at least as large as the fastest rates of the transportation of liquid calculated for such membranes as the gut.[33] We plan to report on this topic in greater detail in the near future.[34]

"*Permselective*" *electronegative membranes* of highest activity and low resistance have been prepared by a variety of methods. The preparation of the original type of these membranes, oxidized collodion membranes, was described in detail.[15, 35] These nearly glass-clear membranes have a thickness of the order of 20 to 40μ, their water content by weight is about 14 to 23 per cent. The resistance of these membranes, in particular, can be adjusted over a wide range by the proper choice of the experimental conditions; *i.e.*, membranes of lower resistance, that is, of greater absolute permeability, have at higher concentrations a somewhat smaller ionic selectivity. The electrochemical properties of these membranes will be discussed later.

The permselective oxidized collodion membranes are of weak acid character. Their mechanical strength, while adequate, is limited due to the process of oxidation to which they were subject. These two points are taken care of by the use of strong acid type polyelectrolytes, such as sulfonated polystyrene, which may either be adsorbed on collodion membranes of high porosity which are dried later, or the sulfonated polystyrene may be incorporated in the collodion solutions from which the membranes are prepared.[17, 18, 36]

Ion exchangers of the commercial type, because of their high charge density per unit area and their low inherent porosity, are obviously a promising material for the preparation of permselective membranes.[2] Having an exchange capacity of several equivalents per liter, membranes prepared from such material can be expected to have a very low resistance, that is, a very high permeability for the critical ions.[2]

By molding under pressure an intimate mixture of powdered cation exchanger of the sulfonic acid type and an inert thermoplastic resin, Wiley and Patnode have prepared rigid discs of 0.5 to 4 mm. in thickness which, at high electrolyte concentrations, show a higher ionic selectivity than the collodion base membranes just described.[37] The resistance of these membranes, at present, is somewhat higher than that of the collodion base membranes, and their equilibration with outside solutions somewhat slower because of their considerable thickness. However, the method of Wyllie and Patnode is open to further improvement. Juda and McRae have announced the preparation of membranes from a sulfonic acid type cation-exchange resin by an unrevealed process.[38, 39] These membranes are commercially available in the form of supported sheets of a thickness of several tenths of a millimeter. They have a high permeability to cations (also a high exchange capacity), but seem to have a considerably lower selectivity than permselective collodion membranes, probably because their porosity has not been adjusted to the optimum. Kressman has briefly described membranes prepared as coherent sheets from a strong acid type ion exchange resin as well as membranes consisting of a supporting material such as paper or cloth which is impregnated with the resin.[19] It appears that the ionic selectivity of these membranes is only moderate, except at very low concentrations. Bonhoeffer, Miller and Schindewolf have described the electromotive properties of chips of strong and weak acid type cation exchanger resin which were used as membranes.[40] Manecke has made a similar study with a cation exchanger resin membrane of an undescribed mode of preparation.[41] These membranes do not appear to have as high a degree of selectivity as the membranes of Wyllie and Patnode or the collodion base membranes.

To what extent the swelling of commercial type, high capacity ion exchangers may be a drawback in their use as membranes, at least for certain purposes, cannot be decided at present. The method of Wyllie and Patnode seems to minimize this difficulty.[37] The high exchange capacity of membranes prepared from commercial type ion exchangers might be a considerable disadvantage for certain scientific purposes. However, this factor would be of no consequence in many technical applications where lowest unit area resistance is of paramount importance. The two main problems in the preparation of highest quality membranes from commercial type ion exchangers without the addition of other materials are the formation of coherent sheets, a topic about which seemingly nothing of significance has been published up to now, and the accurate adjustment of the porosity of such high capacity ion exchanger membranes. There is a wide open field for research and development work in this direction.

"Permselective" electropositive membranes. These were first prepared by the adsorption of protamine on one-, two-, or three-layer collodion membranes of high porosity which are later dried under carefully controlled conditions,[42, 43] the method of their preparation being strictly analogous to that of the permselective oxidized collodion membranes. There is no doubt that all the other

general methods of preparing membranes of high activity which were mentioned before can also be applied to this case provided suitable polyelectrolytes are available. Work in this direction is currently being carried out. Clarke, Marinsky, Juda, Rosenberg, and Alexander[44] have prepared anion exchanger membranes and described their properties in some detail without, however, releasing any information as to the method of preparing these membranes. Generally speaking, they do not approach in selectivity the before-mentioned protamine collodion membranes. The lack of accuracy in the published data does not permit at present a definite evaluation of the over-all quality of these membranes. Manecke and Bonhoeffer have briefly reported the preparation of anion exchanger membranes and investigated their electrical conductance. Their electromotive and selectivity properties seem not to be described as yet.[45] It is only a question of time until permselective anion exchanger membranes of highest quality, as good as or better than the protamine collodion membranes, will be available. The pioneering work of Meyer and collaborators on the synthesis of ion exchanger membranes, basic as well as acid ones, should not be overlooked in this connection.[46]

The Electrochemistry of Permselective Membranes

The subsequent paragraphs try to review in a condensed form some of the systematic investigations which have been carried out with permselective collodion base membranes, the only ones about which data of reasonably high accuracy are available. The other types of permselective membranes follow the same general pattern fairly closely.

The measurement of the membrane concentration potentials consisted of the determination of the electromotive force which arises in the chain electrolyte c_2/membrane/electrolyte c_1, the sign referring to the solution with the concentration c_1, the more dilute solution.

Membranes of ion exchange character in such concentration chains can respond electromotively in a proper manner only if their movable, "critical" ions are the same as those contained in the outside solution. In other words, the membranes, or at least their electromotively functional pores, must be saturated by ion exchange with the critical species of ions under consideration. The periods required for this depend upon the thickness and density of the membrane as well as the nature of the critical ion. They vary from half an hour to several hours with the permselective collodion and collodion base membranes.[47, 48]

If a membrane which is already saturated with a particular species of critical ions is transferred to solutions of different concentration containing the same species of critical ion, its electromotive response in most instances is immediately correct and reproducible, adaptation periods of more than two minutes are rare.[47, 48] This is important in the use of these membranes as practical physicochemical tools, for instance, as membrane electrodes.

The final, stable concentration potential across a given membrane is a function of the nature, the concentration ratio, and the absolute concentrations of the electrolyte in solution. With the exception of the cases in which ideal ionic membrane selectivity prevails, the selectivity of a membrane in its dependence upon absolute concentration is strictly defined only with concentration ratios which differ infinitesimally from unity. Following an established procedure, the concentration ratio 1:2 is used in our more accurate experimental work.[17, 49, 50, *etc.*]

The significance and the physical meaning of membrane concentration potentials, ϵ, can be visualized by reference to its theoretical upper limit, the calculated potential, E_{max}, which would arise if the membrane behaved under a given set of conditions as an ideal machine for the reversible transfer of the critical ion.

The calculations of the theoretically possible maximum values of the concentration potential, E_{max}, are based on well known conventional assumptions concerning the meaning of single ion activities, a topic which cannot be discussed here.[51, 52] The computation of E_{max} is based on the general equations

$$E_{max} = \frac{RT}{z_+F} \, ln \, \frac{a_+^{(2)}}{a_+^{(1)}} \tag{2}$$

and

$$E_{max} = \frac{-RT}{z_-F} \, ln \, \frac{a_-^{(2)}}{a_-^{(1)}} \tag{2a}$$

for negative and positive membranes respectively. z_+ and z_-, respectively, are the valencies of the critical ion. $a_+^{(1)}$ and $a_+^{(2)}$ are the activities $c_+^{(1)} \gamma_+^{(1)}$ and $c_+^{(2)} \gamma_+^{(2)}$ of the cations in solutions (1) and (2); $a_-^{(1)}$ and $a_-^{(2)}$ correspondingly are $c_-^{(1)} \gamma_-^{(1)}$ and $c_-^{(2)} \gamma_-^{(2)}$. In the case of uni-univalent electrolytes the mean activity coefficients were used ($\gamma_+ = \gamma_\pm$; $\gamma_- = \gamma_\pm$). With the uni-bivalent and bi-univalent electrolytes, potassium sulfate and magnesium chloride, the activity coefficient for the univalent critical ions is calculated assuming that their activities are the same as those of the potassium and chloride ions, respectively, in potassium chloride solution of the same ionic strength.

The accuracy of the calculated E_{max} values in the more dilute solutions might be estimated to be of the order of ±0.10 mv. With the highest concentrations the error might be appreciably greater.

TABLE 2 presents the data for potassium chloride and hydrochloric acid chains obtained with an oxidized collodion membrane and a sulfonated polystyrene collodion base membrane. The reproducibility of the individual measurements is here better than ±0.05 mv, except at the highest concentrations of hydrochloric acid. Analogous data for lithium chloride and potassium sulfate measured (±0.1 mv.) across a typical oxidized permselective collodion membrane are presented for easier visualization in FIGURE 2. The broken line represents calculated E_{max} values, the circles are experimental points, the concentrations noted in the figure being the lower concentration in each chain.

TABLE 3 and FIGURE 3 give corresponding data (±0.1 mv.) for four electrolytes and a typical permselective protamine collodion membrane.

The data referring to negative and positive membranes will be considered separately.

The deviation of the experimentally determined concentration potential ϵ

from E_{max} at any given concentration level is a direct measure of the deviation of the membrane from ideality. If we focus our attention first on the oxidized collodion membranes, columns 3 and 6 of TABLE 2, and on FIGURE 2, we see

TABLE 2

CONCENTRATION POTENTIALS ($c_2:c_1$ = 2:1) OF TWO ELECTROLYTES ACROSS A TYPICAL PERMSELECTIVE OXIDIZED COLLODION MEMBRANE AND A REPRESENTATIVE PERMSELECTIVE SULFONATED POLYSTYRENE COLLODION BASE MEMBRANE

(T = 25.00 ± 0.05°C)

1	2	3	4	5	6	7
Concentration of electrolyte solutions $c_2:c_1$	Potassium chloride			Hydrochloric acid		
	Theoretical maximum E_{max}	Concentration potential, ε		Theoretical maximum E_{max}	Concentration potential, ε	
		Oxidized collodion membrane	Sulfonated polystyrene collodion membrane		Oxidized collodion membrane	Sulfonated polystyrene collodion membrane
equiv./liter	*mv*	*mv*	*mv*	*mv*	*mv*	*mv*
0.002/0.001	17.42	17.23	17.28	17.45	17.45	17.45
0.004/0.002	17.34	17.01	17.16	17.34	17.26	17.26
0.01/0.005	17.08	16.97	16.97	17.16	17.01	17.07
0.02/0.01	16.88	16.72	16.72	16.96	16.67	16.89
0.1/0.05	16.30	15.80	16.10	16.76	15.31	16.59
0.4/0.2	16.00	13.85	15.35	17.49	15.66	17.37
1.0/0.5	16.34	10.91	14.56	19.89	17.6	19.1
2.0/1.0	17.33	8.02	13.87	24.38	22.0	23.2

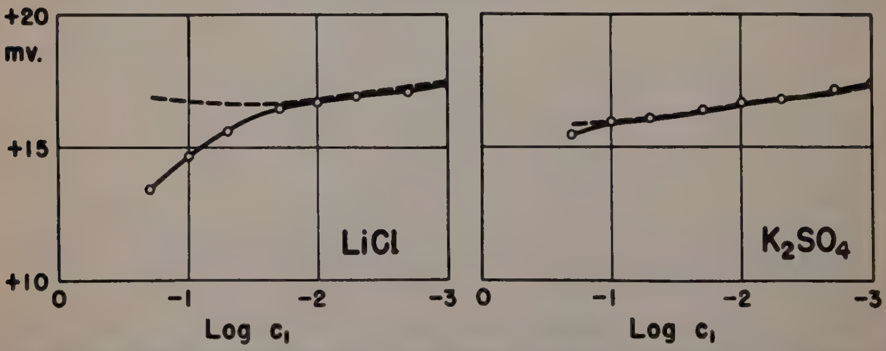

FIGURE 2. Concentration potentials $c_2:c_1$ = 2:1 across a typical permselective oxidized collodion membrane.

that the agreement between the calculated and the experimental concentration potentials is best with potassium sulfate. Here ideal ionic selectivity within the limits of the significance of the data is observed at all concentrations up to 0.2/0.1 N. With potassium chloride, lithium chloride, and hydrochloric acid, these limits are approximately 0.04/0.02, 0.02/0.01, and 0.004/0.002, respectively.

The results with the neutral electrolytes are in best agreement with the theoretical expectation. In the case of potassium sulfate, the bivalent noncritical ion is prevented from penetrating the membrane, both on account of its large size and of the stronger electric repulsion which exists between its double charge and the fixed anionic wall groups. With potassium chloride, the steric factor does not play an important role, since both ions have very nearly the same hydrated size, only electric repulsion being operative. The greater size of the hydrated lithium ion results in a lower degree of ionic selectivity. Some pores which are accessible on a purely steric basis to potassium and chloride ions are inaccessible to the lithium ion.

TABLE 3

CONCENTRATION POTENTIALS ($c_2:c_1 = 2:1$) OF TWO ELECTROLYTES ACROSS A TYPICAL PERMSELECTIVE PROTAMINE COLLODION MEMBRANE ($T = 25.00 \pm 0.05°C$)

Concentration of electrolyte solutions $c_2:c_1$	Potassium chloride		Potassium iodate	
	Theoretical maximum E_{max}	Concentration potential ϵ	Theoretical maximum E_{max}	Concentration potential ϵ
equiv./liter	*mv*	*mv*	*mv*	*mv*
0.002/0.001	−17.4	−16.7	−17.5	−16.2
0.004/0.002	−17.3	−16.8	−17.3	−16.2
0.01/0.005	−17.1	−16.5	−16.9	−16.1
0.02/0.01	−16.9	−16.5	−16.7	−16.0
0.04/0.02	−16.6	−16.2	−16.1	−14.9
0.1/0.05	−16.3	−15.5	−15.1	−12.4
0.2/0.1	−16.1	−14.8	−14.1	−10.2
0.4/0.2	−16.0	−13.5	—	—

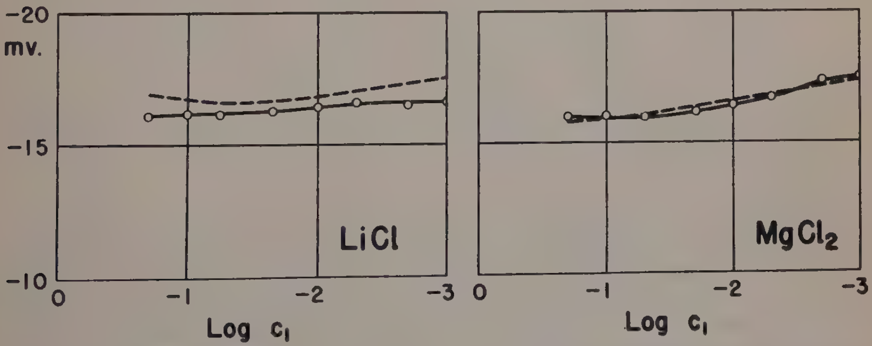

FIGURE 3. Concentration potentials $c_2:c_1 = 2:1$ across a typical permselective protamine collodion membrane.

The behavior of hydrochloric acid, in agreement with the expectation, shows large deviations except at the very lowest concentrations. The oxidized collodion membrane, being of weak acid character, loses at higher hydrogen ion concentrations its characteristic ionic selectivity, it becomes leaky.

The sulfonated polystyrene collodion membrane at low concentration of the neutral electrolyte KCl is just slightly better than the oxidized collodion membrane. At higher concentrations, its selectivity is much higher, however, (column 4 of TABLE 2), as can be expected because of the very high charge density created at the pore walls by the (mono-) sulfonated polystyrene. With the acid solutions (column 7 of TABLE 2) the sulfonated polystyrene membrane, because of its strong acid character, maintains its high selectivity even in fairly high concentrations of acid solutions. As mentioned before, the data of Wyllie and Patnode seem to indicate that their membranes at still higher concentrations are of somewhat superior ionic selectivity.

One might add that unpublished work by Dr. Carr of the University of Minnesota and in our laboratory demonstrates that the sulfonated polystyrene collodion base membranes show a close agreement, at least at low concentrations, between experimental and calculated values in concentration chains involving the alkaline earth metal ions.[53, 54]

The data obtained with permselective protamine collodion membranes (TABLE 3 and FIGURE 3) show that here the agreement between the calculated and the experimental concentration potentials is best with magnesium chloride. Ideal ionic selectivity over wide concentration ranges is approached closely in this case. With the uni-univalent electrolytes, however, an ideal degree of ionic selectivity is not reached in a single instance. Even at the lowest concentrations a considerable difference exists between the measured and the theoretically possible maximum concentration potentials. The membrane selectivity is highest with lithium chloride, less with potassium chloride, and still less with potassium iodate. These differences between the various electrolytes can be readily understood on the same basis as in the case of the electronegative membranes.

The permselective protamine membranes, as we must conclude, have not yet been prepared with the same degree of perfection as that found with the electronegative permselective membranes. The potential data would indicate that they are slightly leaky for univalent noncritical ions. However, other possibilities, too, have to be considered in the explanation of this discrepancy, but our work along these lines has not progressed far enough to make any definite statements.

The resistance data available on permselective membranes are still quite inadequate in scope. Moreover, the method used in the most extensive measurements presented thus far[45, 55, 56] can be criticized because alternating current of a single frequency only was used, while truly reliable and meaningful data can generally result only from carefully designed impedance studies at a great variety of frequencies.[56]

Representative resistance data obtained with a permselective collodion and a permselective protamine collodion membrane of medium resistance between test tube-shaped platinized platinum electrodes at 25.00 ± 0.05°C are listed in TABLE 4. These data, of course, are only illustrative examples to show the differences between different electrolytes. The absolute resistance of the permselective membrane varies greatly depending on minor details in the method of preparation. Collodion base permselective membranes of considerably lower resistance than those shown in TABLE 4 have been prepared, but with this type of membranes some selectivity, particularly at higher concentrations, is lost as the resistance is decreased. Sulfonated polystyrene collodion base membranes of just a few tenths of an ohm resistance per 100 cm.^{2} in 0.1 *N* potassium chloride may show a very high degree of ionic selectivity.

Resistance data on some anion exchanger membranes of considerable ionic selectivity were presented by Clarke, Marinsky, Juda, Rosenberg, and Alexander,[44] and by Manecke and Bonhoeffer.[45]

Closely related to the resistance of the membranes is the rate of ion exchange across them. This rate, under standard conditions, is a convenient measure of ionic membrane permeability. It is also important in the use of the membranes in various physicochemical studies.

If an ideally ion-selective membrane separates two solutions of strong electrolytes, only the "critical" ions exchange across the membrane. If a membrane is not ideally ion-selective, a "leak" of noncritical ions occurs.

TABLE 4

THE ELECTRICAL RESISTANCES OF TWO TYPICAL PERMSELECTIVE COLLODION BASE MEMBRANES IN CONTACT WITH SOLUTIONS OF SEVERAL ELECTROLYTES AT THREE CONCENTRATION LEVELS

Electrolyte solution	Oxidized collodion membrane	Protamine collodion membrane	Electrolyte solution	Oxidized collodion membrane	Protamine collodion membrane
equiv./liter	*Ω/100 cm^2*	*Ω/100 cm^2*	*equiv./liter*	*Ω/100 cm^2*	*Ω/100 cm^2*
KCl			$MgCl_2$		
0.1	2.3	11.8	0.1	96.1	21.1
0.01	4.8	39.3	0.01	148	83.3
0.001	17.6	78.3	0.001	216	353
K_2SO_4			HCl		
0.1	2.4	363	0.1	2.5	—
0.01	6.1	395	0.01	5.1	—
0.001	32.6	457	0.001	21.2	—
K_3-Citrate			K-Acetate		
0.1	2.0	—	0.1	—	63.2
0.01	4.9	—	0.01	—	110
0.001	39.8	—	0.001	—	223
LiCl			KF		
0.1	6.3	16.6	0.1	—	22.5
0.01	11.0	49.8	0.01	—	67.4
0.001	51.3	90.8	0.001	—	136

For the determination of the rates of ion exchange and the leak of noncritical ions the test-tube-shaped permselective membranes (active area about 50 cm.^{2}) were filled with 25 ml. solution of one electrolyte and immersed in a larger test tube containing 25 ml. of solution at the same concentration of some other electrolyte with a different cation and a different anion. Both solutions were stirred, and samples were withdrawn for analyses after measured periods.[35, 43, 57, 59] Representative results obtained with a sulfonated polystyrene collodion base membrane are given in the self-explanatory TABLE 5.[57] Similar results are obtained with protamine collodion membranes, except that a small leak occurs with univalent noncritical ions.

The water permeability of the permselective membranes is low. From the practical point of view it is negligible. For example, a solution of 0.2 *M* sucrose was placed inside a membrane with a resistance of about 1.5 ohms per 100 cm.^{2} in 0.1 N potassium chloride solution. The rate of water movement under these conditions amounted only to 0.01 ml. per 100 cm.^{2} of membrane area per hour.

Some Applications and Uses of Permselective Membranes

Of considerable general and practical interest is the use of permselective membranes as "*membrane electrodes*," which was demonstrated ten years ago,[58] and since then has found a variety of applications in scientific and industrial laboratories. Permselective membranes, by virtue of their ability to act electromotively in the presence of a single species of critical ions like specific reversible electrodes, may be used for the electrometric determination of ion activities in single electrolyte solutions. By their use, it becomes possible to determine (with the before-indicated restrictions) the activities of many ions for which specific reversible electrodes do not exist, as is the case with many anions, F$^-$, NO$_3^-$, Acetate, ClO$_3^-$, ClO$_4^-$, IO$_3^-$, *etc.*, or where specific reversible electrodes of the conventional type involve considerable experimental difficulties, as is the case with the alkali and alkaline earth cations, Li$^+$, Na$^+$, K$^+$, Rb$^+$, Cs$^+$, Mg^{++}, Ca^{++}, *etc.*, and with NH$_4^+$.[37, 41, 58, 59, 60 *etc.*] For cation determinations, the use of the strong acid type sulfonated polystyrene collodion membranes will be preferable in the future over that of the originally used oxidized collodion membranes.

TABLE 5

THE RATE OF CATION EXCHANGE ACROSS TWO TYPICAL PERMSELECTIVE SULFONATED POLYSTYRENE COLLODION MEMBRANES

Resistance of membrane in 0.1 N KCl	Original solutions		Cation exchange after one hour	Anion "leak" after one hour	Cation exchange after four hours	Anion "leak" after four hours
	Inside	Outside				
Ω/100 cm.^{2}	*equiv./liter*	*equiv./liter*	*per cent of possible exchange*	*per cent of actual cation exchange*	*per cent of possible exchange*	*per cent of actual cation exchange*
0.6	0.1 NH_4Cl	0.1 $LiNO_3$	35.0	not detectable	83.0	<0.5
0.9	0.1 NH_4Cl	0.1 $LiNO_3$	29.0	not detectable	68.5	<0.5
0.6	0.1 $(NH_4)_2C_2O_4$	0.1 Li_2SO_4	35.0	not detectable	79.5	<0.05
0.9	0.1 $(NH_4)_2C_2O_4$	0.1 Li_2SO_4	29.5	not detectable	73.0	<0.05

The determination of ion activities by means of the permselective "membrane electrodes" may be made in various ways. The potential difference which arises between a known solution on the one side of the membrane and the solution of unknown concentration on the other side of the membrane may be evaluated on the basis of some calculated standard curve; or it may be compared to an empirical curve determined for a specific membrane in advance. A third method consists of an electrometric titration: the membrane separates the solution of unknown concentration from water, to which electrolyte solution of known (higher) concentration is added stepwise; thereby, the potential difference is diminished stepwise and finally it is reversed. Zero potential difference indicates that the activity of the critical ion is the same on both sides of the membrane. The zero point is obtained conveniently and with considerable accuracy by plotting potential versus concentration data. The titration method makes it possible to minimize the uncertainties of the asymmetry of the liquid junction potentials arising from the use of nonspecific (*e.g.*, calomel) electrodes. It also is less sensitive towards slight "leaks" of the membrane than the two other methods. With membranes which are already saturated with the critical ions under investigation, activity determinations can be made within a few minutes. Results with an error of less than ±1.0 per cent may now routinely be obtained in wide concentration ranges. The limits of this method, which is readily applicable on the semi-microchemical and microchemical level, have not yet been explored.*

The theoretical investigation of the *Gibbs-Donnan membrane equilibrium* has in the past outrun its experimental study. Extensive theoretical discussions of Gibbs-Donnan membrane equilibria can be found in the literature. Their experimental study, however, was confined in the past to systems containing colloidal or semi-colloidal ions, as nondiffusible ions, and a few systems in which the ferrocyanide ion acted as the nondiffusible ion in conjunction with a copper-ferrocyanide membrane.[63, 64, 65] Except for this latter case, Donnan equilibria involving only strong inorganic electrolytes, in which relatively small ions act as "nondiffusible" ions, could not be studied. Suitable membranes for such investigations were nonexistent. Now, however, the permselective membranes lend themselves admirably to this purpose.[59, 66]

In order to demonstrate the existence of a membrane equilibrium across a certain membrane it is only necessary to test experimentally the classical Donnan equations. For the case of two uni-univalent electrolytes $A^+ C^-$ and $B^+ C^-$ and a membrane permeable for the cations A^+ and B^+ and impermeable for the anion C^- (such as the permselective collodion membranes) the Donnan equation, written in a convenient manner, reads:

$$\frac{a_{A^+}^{(1)}}{a_{B^+}^{(1)}} = \frac{a_{A^+}^{(2)}}{a_{B^+}^{(2)}} \qquad (3)$$

where a is the activity of the respective ions in solutions (1) and (2). If the experimental system is selected so that the ions A^+ and B^+ have the same activities in solutions of the same ionic strength, then the ratios of the activity coefficients will be unity and one may use concentrations, c, instead of activities, EQUATION 3 becomes the expression

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* It must be pointed out here that Marshall and collaborators have prepared and investigated clay membranes which may be used as membrane electrodes for the determination of the activities of univalent, and in some instances also of bivalent cations.[61] These membranes, as it seems, are difficult to prepare, have a very high resistance and therefore require long periods of adaptation before final, reproducible potentials are established. When in use, their durability is limited. The range of highest ionic selectivity is comparable to that of the best permselective collodion base membranes. Marshall's work, particularly his thoughtful theoretical considerations concerning the electrochemistry of membrane electrodes, deserves the most careful consideration of anyone interested in this field. The detailed discussion of Marshall's very interesting membranes is outside the scope of this review, particularly since Marshall seems now inclined to consider them rather as solid electrolyte phases than as membranes of porous character as this term is used here. In the same category as Marshall's clay membranes are the membranes cut from natural shales which Wyllie has described.[62]

$$\frac{c_{A^+}^{(1)}}{c_{B^+}^{(1)}} = \frac{c_{A^+}^{(2)}}{c_{B^+}^{(2)}} \tag{3a}$$

The theoretically predicted equilibrium conditions hold true if the ratio of the analytical concentrations of the two ions of both sides of the membrane, as determined by analytical procedures, is identical (and if the same final condition is reached independent of the original distribution of the diffusible ions between the two solutions).

EQUATION 3a was tested with the same experimental arrangement as described previously for the studies on the rate of ion exchange. Osmotic equilibration can be readily established by the addition of the proper amount of a nondiffusible nonelectrolyte (sucrose) to the more dilute solution, though this is not necessary with numerous experimental systems, on account of the low water permeability of the permselective membranes which was referred to before.

TABLE 6

GIBBS-DONNAN EQUILIBRIA ACROSS PERMSELECTIVE COLLODION MEMBRANES WHICH INVOLVE ONLY STRONG INORGANIC ELECTROLYTES (THE ANIONS ARE THE NONDIFFUSIBLE IONS)

Ratio of volumes of $\frac{\text{Solution in}}{\text{Solution out}}$	Solute	Original state millimoles/liter		Equilibrium state				Concentration ratio $\frac{\text{In}}{\text{Out}}$	
				Experimental millimoles/liter		Calculated millimoles/liter			
		In	Out	In	Out	In	Out	Experimental	Calculated
1:1	NH_4^+	20.0	10.0	22.4	7.5	22.5	7.5	2.99 ± 0.05	3.00
	K^+	10.0	—	7.4	2.4	7.5	2.5	3.08 ± 0.10	3.00
	Cl^-	30.0	10.0	30.0	10.2	30.0	10.0	2.94 ± 0.05	3.00
	Sucrose	—	33	—	(33)	—	(33)	—	—
1:1	NH_4^+	30.0	—	22.4	7.5	22.5	7.5	2.99 ± 0.05	3.00
	K^+	—	10.0	7.5	2.5	7.5	2.5	3.00 ± 0.10	3.00
	Cl^-	30.0	10.0	29.8	10.1	30.0	10.0	2.95 ± 0.05	3.00
	Sucrose	—	33	—	(33)	—	(33)	—	—
1:10	NH_4^+	50.2	2.51	37.4	3.79	37.5	3.78	9.9 ± 0.3	9.9
	K^+	—	2.56	12.0	1.27	12.7	1.29	9.4 ± 0.4	9.9
	$C_2O_4^-$	25.1	2.54	24.7	2.53	25.1	2.54	9.8 ± 0.2	9.9
	Sucrose	—	39	—	(39)	—	(39)	—	—

The Donnan membrane equilibrium was established in the various systems in three to 24 hours according to the nature (resistance) of the membrane used. The results of three typical experiments on membrane equilibria across permselective collodion membranes are given in the self-explanatory TABLE 6. The ratio of the activity coefficients of the K^+ and NH_4^+ salts used in the pairs of solutions given in this table are nearly identical.

Preliminary tests have shown that protamine collodion membranes are also usable for Donnan experiments.

The significance, for the pure physical chemistry of electrolyte solutions, as well as for the study of colloidal systems, of the possibility of the experimental study of membrane equilibria which may involve almost any desired combination of uni-univalent and many combinations of uni-polyvalent strong electrolytes, hardly requires any emphasis. Work along these lines is currently under way. Such studies will become increasingly fruitful as they can be extended in the direction of more and more concentrated electrolyte solutions, as membranes with a larger range of virtually ideal ionic selectivity become available.

The experimental study of Donnan equilibria involving only the ions of strong inorganic electrolytes by means of the permselective membranes and the use of the latter as "membrane electrodes" has furnished a lead to the solution of an old and vexing electrochemical problem.[67] Heretofore, the *activities of several coexisting species of ions* of the same sign in solution could be determined unequivocally only if specific electrodes, which are lacking for many common ions, could be used. Attempts to overcome this difficulty indirectly, *e.g.* by "compensation dialysis," have been only indifferently successful. The Gibbs-Donnan membrane equilibrium principle, however, permits an exact and general solution of this problem which is not restricted by the lack of specific electrodes. It is based on a consideration of the ion distribution *and* of the concomitant membrane potential.

A solution I contains the anions R^- and NO^-_3, and the cations K^+, Na^+, and NH_4^+, the activities of which $[a_{K^+}]_I$, $[a_{Na^+}]_I$, and $[a_{NH4^+}]_I$ have to be determined. A (permselective) membrane permeable to all cations and impermeable to all anions separates solution I from a KCl solution II of known concentration. Solution II is iso-osmotic with I, or is made iso-osmotic by the addition of nondiffusible nonelectrolyte. The volume of solution I is made infinitely larger than that of II so that the composition of I is not changed significantly after equilibration with solution II.

After the system is equilibrated the membrane potential ϵ is measured and solution II analyzed. The final state of the system is

$$[K^+]_I\,[Na^+]_I\,[NH_4^+]_I\,[R^-]_I\,[NO_3]_I \,\|\, [K^+]_{II}\,[Na^+]_{II}\,[NH_4^+]_{II}\,[Cl^-]_{II}.$$

The activities in II of the cations $[a_{K^+}]_{II}$, $[a_{Na^+}]_{II}$, and $[a_{NH4^+}]_{II}$ are calculated from the known analytical concentrations according to the Debye-Hückel theory.

The Gibbs-Donnan theorem postulates for the ion distribution

$$\frac{[a_{K^+}]_I}{[a_{K^+}]_{II}} = \frac{[a_{Na^+}]_I}{[a_{Na^+}]_{II}} = \frac{[a_{NH_4^+}]_I}{[a_{NH_4^+}]_{II}} \tag{4}$$

and for the membrane potential

$$\epsilon = \frac{RT}{F}\,ln\,\frac{[a_{K^+}]_I}{[a_{K^+}]_{II}} = \frac{RT}{F}\,ln\,\frac{[a_{Na^+}]_I}{[a_{Na^+}]_{II}} = \frac{RT}{F}\,ln\,\frac{[a_{NH_4^+}]_I}{[a_{NH_4^+}]_{II}} \tag{5}$$

The unknown activities in solution I of the cations $[a_{K^+}]_I$, $[a_{Na^+}]_I$, and $[a_{NH4^+}]_I$ can be evaluated from EQUATION 5.

This method is, of course, not restricted to the simple conditions illustrated here, but it may be extended to more involved ionic systems. It will furnish considerable help in the solution of numerous problems in colloid chemistry, physiology, and biochemistry.

Whereas the membrane potentials which arise in equilibrium systems (and quasi-equilibrium systems) are clearly and quantitatively understood on the basis of the theory of the Donnan membrane potential, no general statement can be made concerning the *dyamic membrane potentials* which arise in systems which drift toward an equilibrium.

The simplest possible case of a dynamic membrane system with more than one species of potential determining ions, aside from the trivial case of concentration chains with membranes of less than ideal ionic selectivity, is the type of chains in which the so-called *bi-ionic potentials* arise.

The bi-ionic potential, B.I.P., has been defined as the dynamic membrane potential which arises across a membrane separating the solutions of two electrolytes at the same concentration with different "critical" ions, which are able to exchange across the membrane, and the same "noncritical" ion species for which the membrane is (ideally) impermeable.[68]

The general scheme of a chain in which a B.I.P. arises across an electronegative membrane can be represented in the following manner:

Solution 1		*Solution* 2
Electrolyte A^+C^-	Electronegative Membrane	Electrolyte B^+C^-
c_1		c_1

A^+ and B^+ represent (univalent) cations which are able to exchange freely across the membrane, and C^- an anion which is unable to penetrate through it.

Systems of this type have been investigated experimentally only in a few isolated instances. Bi-ionic potentials, according to the nature of the membrane and the combination of critical ions, may be as high as 150 millivolts, fairly independent of the absolute concentration. The membrane has a rather unpredictable influence on the absolute magnitude of these potentials. There are, however, two consistent and characteristic sequences in which the various cations and anions can be arranged according to the relative magnitude of the bi-ionic potential which is caused by their presence. These two ionic sequences are identical with the so-called Hofmeister series.[69, 70]

A satisfactory explanation of the mechanism of the origin of the bi-ionic potential cannot be looked for, as was suggested by Michaelis and Fujita,[71] in the difference of the size of the various ions and their concomitant differential exclusion from the pores of the membrane, since later data[69, 70] show no correlation between the hydrated size of the various ions and their electromotive efficiency in bi-ionic potential systems.

From the formal electrochemical point of view, as was pointed out by Michaelis, the sign and the magnitude of the B.I.P. depends on the relative ease with which the two species of critical ions can penetrate across the membrane.[3, 71] The more readily permeable critical ions impress a potential on the other solution which is identical in sign with that of their own charge. A formal quantitative evaluation of the B.I.P., which will be omitted here, can be based on the general concept of transference numbers, in analogy to that which was applied previously to the concentration potential.

A satisfactory solution of this long controversial problem was arrived at by the consideration of the membranes as ion exchange bodies according to the fixed charge theory.[68, 72] The two exchangeable (critical) species of ions compete for positions as counter-ions of the fixed dissociable groups of the membrane. Concerning the general phenomenon of competitive ion exchange it is well known from extensive studies in ion exchange that the various competing ions are taken up by ion exchangers to a very different extent. The kinetics of such processes in general have been considered by Jenny and others.[73, 74] The sequences of their relative adsorbabilities are roughly the two Hofmeister series of cations and of anions unless steric hindrance and related factors come into play as complicating factors. The adsorbed critical ions are dissociated off in the pores according to their nature and the nature of the fixed ionic wall groups.

The relative abundance of the two species of critical ions in the pores multiplied by their diffusion velocities and valencies determine their respective contributions toward the virtual transportation of electricity across the membrane, which decide the sign and the magnitude of the B.I.P. Worked out in detail, this theory of the origin of the B.I.P. seems to fit all the known experimental data.

The elucidation of the mechanism of the bi-ionic potential is of particular interest here because it demonstrates that the consideration of the membranes as ion exchangers, according to the fixed charge theory, opens up to rational study the wide field of *polyionic membrane systems* in general. Current work[75] shows that the same basic considerations which were essential in clarifying the simple case of the B.I.P. can be extended, not only to instances where two critical ions are present in different ratios at the two sides of the membrane, that is, to degrading B.I.P. systems, but also to cases where the absolute concentrations at the two sides of the membrane are not the same. Analogous systems containing more than two species of critical ions and even somewhat leaky systems seem amenable to a theoretical treatment which can be verified by the experimental test.[75] The importance of this possibility for the elucidation of a great variety of bio-electrical phenomena does not require any special comment.

The use of permselective membranes in preparative chemistry and in industrial operations for the purpose of the exchange, between solutions, of ions (including the exchange of the ions of water) is, at present, beginning to be explored. It might prove to be of great practical significance that ion exchange operations might be performed between phases of different water activity without a disturbing extent of osmotic solvent movement. Industrial chemists will gradually become interested in the numerous possibilities in this field.

To give just a few examples. The salts of pH sensitive organic acids may be converted into the free acid either by membrane hydrolysis or by dialytic ion exchange against an acid without ever coming into contact with the latter as occurs in precipitation reactions.

The regeneration of certain industrial waste liquids seems feasible. For instance, in the system: fairly concentrated (c_1) solution of sodium sulfate/negative permselective membrane/dilute (c_2) sulfuric acid, ($c_1 \gg c_2$), the hydrogen and the sodium ion would exchange across permselective membranes without significant movement of water. In a counter current system, the result would be a sulfuric acid solution of approximately the higher (useful) concentration, c_1, and a waste solution of sodium sulfate of the lower concentration, c_2.

Nonelectrolytes of medium, and even of low molecular weight might be separated from electrolytes by the simultaneous use of both electropositive and electronegative membranes in diffusion-dialysis; or ion exchange dialysis against outside solutions of an acid and a base, respectively, might be employed for the same purpose in a three-cell outfit; or, most promising, electrodialysis might be used to accelerate the speed of electrolyte removal from the middle cell.[2] This latter possibility has recently attracted considerable industrial and popular interest. The use of permselective membranes of necessity eliminates various complications in electrodialysis, several of which are too involved to be taken up here. The movement to the middle cell of anions from the cathode compartment and of cations from the anode compartment can be eliminated by use of permselective membranes. Outside solutions of considerable conductance thus become feasible. Their use would greatly increase the efficiency of the process. Thus, the use of permselective membranes can be hoped to be the decisive step which, at long last, might make electrodialysis an economically attractive industrial method, after it has been lying more or less dormant for several decades.

The separation of bivalent and monovalent ions of the same sign of charge by means of permselective membranes of carefully adjusted porosity seems to be a problem of some interest.

The discussion of the application of permselective membranes would be incomplete without reference to at least one example of their usefulness in various *model studies of biological significance.*

The literature contains a great variety of discussions of the possible or probable role of membranes in many complex *in vivo* processes which involve electrolytes. The experimental data in this direction are in an extremely unsatisfactory state, primarily because of the formerly prevailing lack of adequate membranes.

We have recently investigated one such complex system involving the much discussed situation of a membrane composed of preferentially anion and preferentially cation permeable parts.

Such so-called *mosaic membranes* have been shown to be amenable to a very simple quantitative theoretical analysis from the electrochemical point of view provided the membranes are of an ideal degree of ionic selectivity, or nearly so.[76]

Let us consider a system composed of two potassium chloride solutions of different concentrations which are separated by a mosaic membrane composed of ideally anion selective and ideally cation selective parts. Contrary to certain statements in the literature, it is immediately evident that the concentration difference between the two solutions will become equalized in the course of time, anions moving through the anion permeable parts of the membrane and cations through its cation permeable segments.

The quantitative analysis of this situation can be based on an imaginary separation of the anion and the cation permeable membrane parts. This results in a ring system which represents an "all electrolytic fluid circuit" ("Flüssigkeitsring") in the sense of Dolezalek and Krüger,[77] composed of the following cyclic arrangement: concentrated solution/positive membrane/dilute solution/negative membrane/, with the negative membrane in turn in contact with the concentrated solution.

The two electromotive forces arising at the two membranes in this ring system are arranged in series. Their magnitude can be measured or calculated from the known activities of the two solutions. The total resistance of the system can be determined experimentally. With the total electromotive force and the resistance of the system known, the strength of the current which flows in it can be calculated.

It is immediately evident that the quantity of electricity which is transported by this current in a given time, expressed in Faradays, must be numerically identical with the equivalents of electrolyte which have moved during the same period from the concentrated to the dilute solution. The all electrolytic ring system, in the language of conventional electrochemistry, is nothing but two (membrane) concentration chains arranged in series.

The electrolyte permeability of mosaic membranes composed of ideally anion and ideally cation permeable parts can thus be precalculated quantitatively on the basis of purely electrical data.

The experimental test of this theory involves a considerable amount of careful laboratory work. Without going into the details, it seems sufficient to report here that quantitative agreement with the predictions of the theory was found, the ratio of the number of Faradays of electricity moved and the number of equivalents of electrolyte transported across the membranes is 1:1 with an average error of ±2 per cent.[36, 54, 78] This seems to be the first instance in which a complex membrane system was amenable to such quantitative treatment.

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THE SEPARATION OF BIOCHEMICALLY IMPORTANT SUBSTANCES BY ION EXCHANGE CHROMATOGRAPHY*

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Although ion exchange, as a chemical reaction, is over a century old and ion-exchange reactions (*e.g.*, water softening) have been commonplace in chemical technology for decades, it has been only a few years since the first demonstration of ion-exchange chromatography of a precision capable of separating the closely related members of such families as the rare earths and the amino acids. With these demonstrations, which could not have taken place before the invention of the modern, stable, reproducible, synthetic exchangers, ion-exchange chromatography has emerged as a powerful analytical and preparative tool with which to approach those biochemical separation problems which had yielded only partially or not at all to classical methods. It is in the separation of each of the individual members of the conventional biochemical mixtures that ion exchange has made and promises to make its greatest contributions to biochemistry, contributions which far exceed in general utility the older applications to the isolation of single substances or of groups of related substances.

Other papers in this monograph deal with basic principles and nonbiochemical or nonchromatographic applications of ion exchange. This paper is limited to an exposition of the more recently reported chromatographic ion exchange separations of the members of families of biochemical importance, specifically, amino acids, purine and pyrimidine bases, nucleotides, sugars and sugar derivatives, carboxylic acids, and proteins. No attempt at comprehensive historical development or bibliographic citation is made. For this the reader is referred to the report by Cannan[1] before this Academy in 1946 and to the review by Block[2] in 1949, both concerning the ion exchange properties of amino acids; to those of Applezweig[3] and of Applezweig and Nachod[4] dealing with general biochemical and physiological applications and separations; and to the more recent comprehensive reviews of Kunin[5] and of Kressman.[6]

General Considerations

The most useful method for the separation of components in biochemical mixtures seems to be that of "elution analysis." This is a two-step process, wherein the mixture is first adsorbed at the top of a column, previously prepared in a given form, and then eluted in such a manner as to bring each substance to the bottom of the column as a separate and distinct "peak" without significant change in the form of the exchanger (in contradistinction to "displacement chromatography" where a change in the form of the column does take place). In general, the adsorption step is carried out under conditions where the affinities of the ions in question for the exchanger are maximal, or at least greater than during the elution sequence. Elution utilizes conditions

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which decrease the affinities, releasing the ions from the exchanger. In so far as the adsorption is due to ionic forces,* elution conditions fall into two main groups, (1) simple increase in the concentration of competing ion to the point where it displaces the adsorbed ions by mass action (ionic-strength adjustment) and, (2) change (usually decrease) in the charge of the adsorbed ion by pH change, complex formation, the use of a nonpolar solvent, *etc.* (charge adjustment).

The method of charge adjustment is obviously applicable only to ions with variable charge, a not unusual situation in biochemistry. Amino and carboxyl groups are readily ionized or deionized, phosphate esters become singly or doubly ionized, sugars form borate complexes, *etc.*, at pH values that are not difficult to attain in the laboratory and usually are not destructive to the material under investigation. Furthermore, elution by charge adjustment makes it possible to recover the ions in question in solutions of lower ionic strength and thus of greater flexibility with respect to subsequent chemical steps. These features, together with the inherently greater flexibility in the separations themselves, have made elution by charge adjustment the method of choice in most fields so far investigated.

Amino Acids

In reviewing his pioneer experiments in amino acid chromatography and those of Wieland; Englis and Fiess; Turba and Richter; Cleaver, Hardy and Cassidy; Block; and Freudenberg *et al.*, before the New York Academy of Sciences in 1946, Cannan[1] remarked, "By reason of high capacity, uniformity of product, stability, and long life the ion exchange resins are attractive analytical agents. The experiments quoted above suggest that they may have some value in the analysis of mixtures of amino acids." The more recent displacement development procedures of Partridge and Brimley in England and the elution analysis schemes developed by Stein and Moore in this country represent a full realization of this prediction.

The earlier separations of amino acids by Partridge and his colleagues,[10-12] utilizing displacement chromatography on the weak-base and weak-acid exchangers and on the sulfonated phenol-formaldehyde resins containing free phenolic groups, were only partially successful if the ultimate objective is considered to be the separation of each component in a single experiment. Although separation of many constituents was realized, others were obtained in groups. Davies,[13] in his discussion of the factors involved in the separation of weak electrolytes on ion exchangers, points out the disadvantages of the displacement technique in this situation, in which rapid equilibration of exchanger and resin is not achieved. The more recent experiments of Partridge and Brimley,[14, 15] utilizing polystyrene exchangers and noting the effect of temperature, confirm the practical importance of eliminating weak exchange groups and also demonstrate the contribution made by non-ionic forces to the

* The solubility of the organic portion of many ions in the organic matrix of the exchanger must not be forgotten as a contributing condition, one which accounts for many aberrations from predictions based on charge alone. Chromatography and adsorption on ion exchangers in which such nonionic forces predominate are described by Weiss,[7] Davies and Thomas,[8] and Cleaver and Cassidy.[9] The influence of such forces is discussed in connection with the specific separations described in the following sections.

affinity of an amino acid for the exchanger matrix. These experiments result in an order of elution of amino acids which Davies[13] shows to be consistent with both the ionic and nonionic properties of the several components (*cf.* Cleaver and Cassidy[9]).

Although displacement chromatography has been largely supplanted in popularity by elution analysis (see the following discussion), several investigations involving both normal and unusual amino acids or their analogues have made use of the method.[16-18]

For the separation of amino acids by elution analysis, either in one experiment or in consecutive experiments, four possibilities exist: a cation exchanger in either the acid or the salt form (with elution by acid or salts, respectively); or the use of an anion exchanger in either the base or the salt form (with elution by base or salts, respectively). Anion exchange has not been popular in spite of the early separation of glutamic and aspartic acids on a weak-base exchanger by Consden, Gordon, and Martin[19] and the theoretically satisfactory but practically difficult displacement experiments of Davies, Hughes, and Partridge[20] on a strong-base anion exchanger. This lack of popularity is perhaps due as much to the potential influence of nitrogen loss from the exchanger upon nitrogen analyses of the eluates as to the degree of success attending elution analysis on cation exchangers (see the following discussion). Nevertheless, until there has been a thorough trial of elution analysis on strong-base anion exchangers for comparison, it remains to be seen what, if any, advantages are being overlooked in this direction.

In FIGURES 1 and 2 are shown the two highly successful procedures developed by Stein and Moore[21, 22] for the elution analysis of a mixture of amino acids by cation exchange. FIGURE 1 shows a separation of the amino acids encountered in the usual protein hydrolysates by hydrochloric acid from a sulfonated polystyrene resin.[21] Only a partial separation of serine, threonine, and glutamic acid is realized. Other undesirable features apparent are the high concentration of hydrochloric acid needed and the poor definition of certain peaks.

Utilizing the sodium form of the same exchanger, eluting with sodium buffers of continually increasing pH, and varying the temperature whenever necessary to effect or to improve a separation, Stein and Moore[22] have achieved a better separation of the same components represented in FIGURE 1 plus a variety of related substances. The procedure is shown in FIGURE 2.

Although there is unquestionably an exchange of hydrogen or sodium ions, respectively, for amino acids in these two experiments, ionization of the acids is not the only factor entering into the distribution coefficient which determines the position of each component in the chromatogram. Glycine, alanine, valine, isoleucine, and leucine have nearly the same pK_{B1} values (4.22, 4.13, 4.28, 4.24, and 4.25, respectively) and pI values (6.06, 6.11, 6.00, 6.04, and 6.04, respectively), yet they show a variable degree of separation in each instance even though the order is the same, which may indicate that the same complex of factors is operative in each. Further evidence that nonionic forces are operative is found in the positions of phenylalanine and tyrosine, which have pK_{B1} values (4.76 and 3.93) and pI values (5.91 and 5.63) in the same range as those given for the aliphatic amino acids and in the relatively greater decrease in affinity of the more nonpolar members brought about by nonpolar solvents.[22] The operation of these forces in amino acid separations on ion exchangers is discussed by Davies[8, 13] and by Cleaver and Cassidy.[9]

A successful application of these principles to the separation of di- and tripeptides has been made by Brenner and Burkhardt.[23]

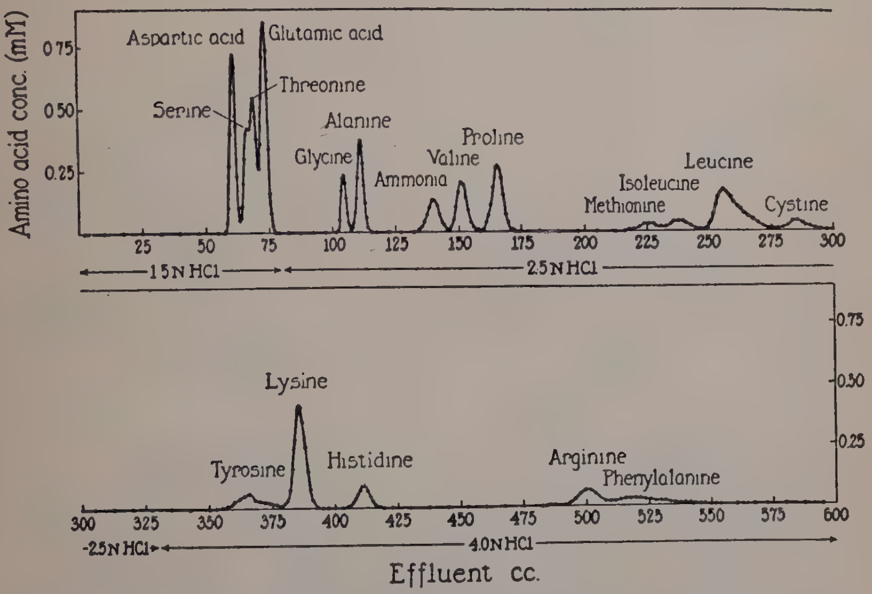

FIGURE 1. Fractionation of a known mixture of amino acids on Dowex-50. Solvents, 1.5N, 2.5N, and 4.0N HCl. Column dimensions, 0.9 x 55 cm. Sample, about 3 mg. of amino acids. Stein and Moore.[21]

Nucleic Acid Derivatives

Nucleic acids may be degraded to their purine and pyrimidine bases, and the quantitative analyses of mixtures of these substances have occupied the attention of many investigators ever since their discovery. These substances, although classified as bases, are capable of functioning either as acids or bases although uracil and thymine have essentially no basic properties and cytosine and 5-methylcytosine have essentially no acidic properties. The other three heterocyclic compounds can be adsorbed and eluted from either anion exchangers or cation exchangers.

The separation of these bases by simple ion replacement from a cation exchanger is shown in FIGURE 3. Thymine, which has negligible cationic properties, would appear with uracil. The order of the other three bases, predicted from the amino-group dissociation constant, is guanine, adenine, and cytosine. The aberration in the position of cytosine found is consistent with the family difference between purines and pyrimidines noted below, which is ascribed to a non-ionic attraction of the purines enhancing their total affinity for the exchanger.

As anions, these five bases are separable as shown in FIGURE 4 by charge adjustment in a chloride (or formate) system.[24, 25] Here, there are at least two aberrations from the order of the acidic dissociation constants: adenine should appear with guanine; thymine (5-methyluracil) has a higher pK value than uracil and should precede it. In the latter case, the addition of the methyl group may explain the additional affinity which reverses the predicted order.

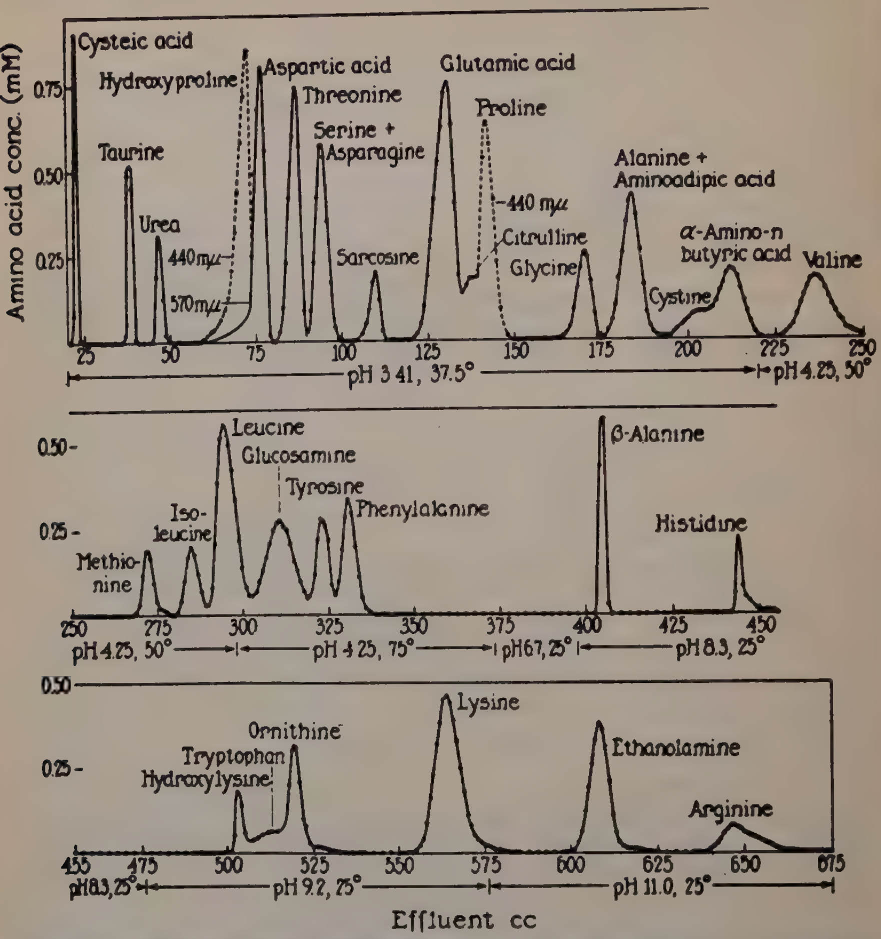

FIGURE 2. Elution of amino acids and related compounds (approx. 10 mg. total) from 0.9 cm. x 100 cm. sodium form, Dowex-50 column with sodium buffers of about 0.2 *M* sodium ion concentration at about 4 cc. per hour. Stein and Moore.[22]

The ribosides and desoxyribosides of these bases partake of their behavior, with the exception of adenosine, in which esterification of position 9 of adenine by ribose removes the most effective acid group of adenine and decreases markedly its absorption by anion exchangers. Separations of uridine and cytidine by cation exchange absorption of the latter have been reported by

Elmore[26, 27] and by Harris and Thomas.[28] A cation exchange separation of four desoxynucleosides, utilizing ammonium acetate elution from a sulfonic acid cation exchanger in the ammonium form, has been utilized by Reichard and Estborn[29] in the preparation of these substances. The volatility of ammonium acetate makes its removal very simple. The same substances have been separated recently by Andersen, Dekker, and Todd[30] by anion exchange procedures similar to those described by Cohn[31] but with the addition of a carboxylic acid exchange step to separate cytidine and adenosine. Elution of these was accomplished with water (hydrolysis) with cytidine preceding adenosine. This absorption-elution sequence seems to be governed by non-ionic properties as the values of the respective basic dissociation constants would predict the reverse order of elution. This is similar to the increase in affinity of purine over pyrimidine compounds which has been noted for the bases and is discussed in the following for the nucleotides.

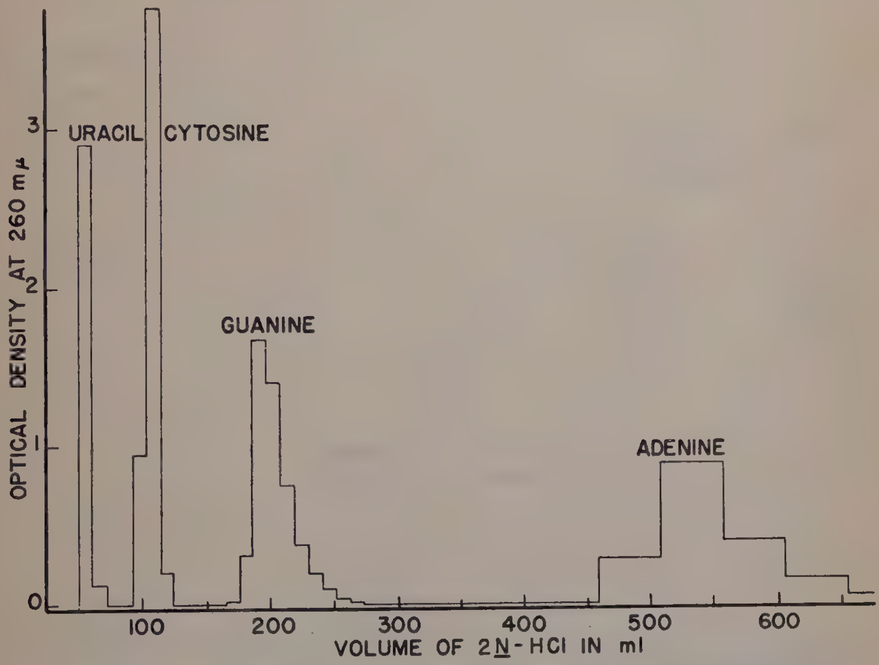

FIGURE 3. Separation of purine and pyrimidine bases by a cation exchange column (Cohn[24, 25]). Resin: Dowex-50, ν 300 mesh, H^+ form. Bed: 81 mm. x 0.74 cm.^{2} Rate: 0.6 ml/min. Eluting solution: 2 N—HCl. Test material: 0.5 mg. uracil, 1.0 mg. each of cytosine, guanine, adenine in 7.5 ml. 2 N—HCl. Recoveries: 98.5–101.5 per cent (based on optical density at 260 mμ).

The degradation of nucleic acids to nucleotides, in which the above bases remain linked to ribose or desoxyribose which is, in turn, esterified with phosphate, results in a mixture of substances with properties which are such as to make separations by the classical techniques of precipitation very difficult. The pK values of the phosphoric acid groups[32] are quite similar, but the larger variation in the pK values of the bases results in a considerable variation in the net charge per molecule as a function of pH, as shown in FIGURE 5. It is apparent that the net charges vary most in the region of pH 2–3 and it is in this region that the first separations of these substances were made.[24, 25, 31]

An examination of these curves indicates that elution from an anion exchanger at pH 4 should bring adenylic off ahead of uridylic if net charge is the sole criterion. Similarly, at pH 2, guanylic should precede uridylic. Actually, at pH 4 uridylic precedes adenylic and, at pH 2, appears simultaneously with guanylic. It is thus clear that the purine compounds are, in general, more strongly bound than the dissociation constants alone would predict, again bearing out the hypothesis that non-ionic forces contribute to the total affinity observed.[31] Nevertheless, the order in the pH range 2.5–3.0 is cytidylic, adenylic, and guanylic, which is the order of the dissociation constants of the amino groups (pK = 4.2, 3.7, and 2.3 respectively),[32] which in turn determine net charge in this range.

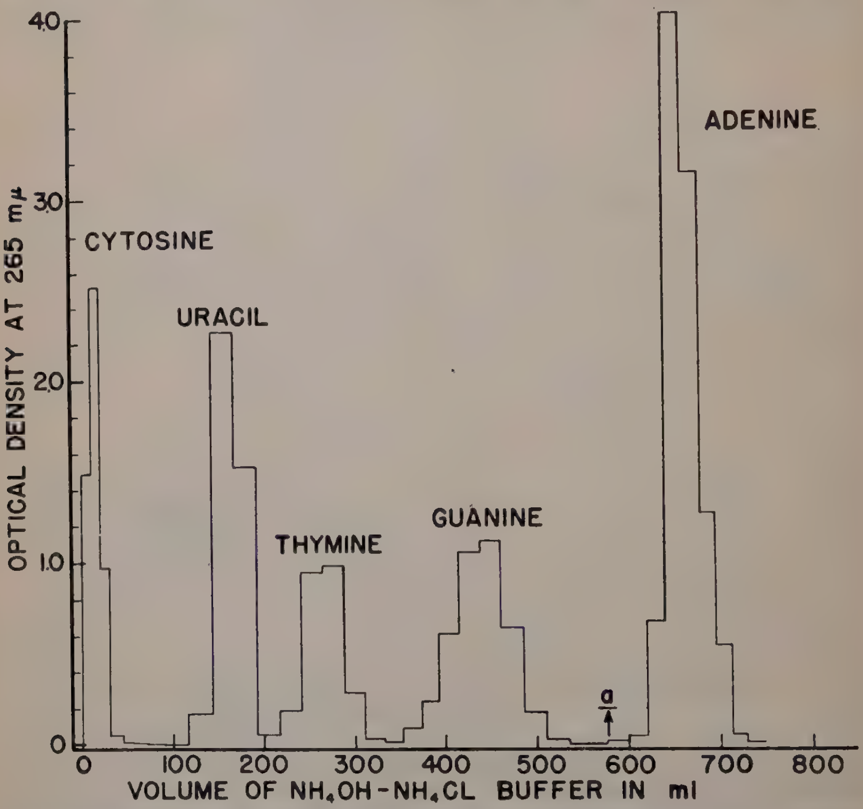

FIGURE 4. Separation of purine and pyrimidine bases by an anion exchange column (Cohn[24, 25]). Exchange Dowex Al ν 300 mesh, Cl^- form. Bed: 85 mm. x 0.74 $cm.^2$ Rate: 0.25 ml/min. Test material: 1 mg. cytosine 2 mg. each of other bases in eluant solution. Eluting solution: 0.2 M—NH_4OH-NH_4Cl buffer, pH 10.6, 0.025 MCl^- (at *a*, pH → 10.0, Cl^- → 0.1 M). Recoveries: 97.5–99 per cent (based on 265 mμ densities).

The application of this technique for nucleotide analysis to the unpurified mixtures obtained from the hydrolysis of native nucleic acid led to the discovery[31, 33] of two isomeric forms of each nucleotide, see FIGURE 6 (b). One of these, the *b* form in each instance, was identical with the substances described earlier by Levene and his collaborators.[32] In each case so far measured, it is the more insoluble of the pair and has the lower pK value. Since it had

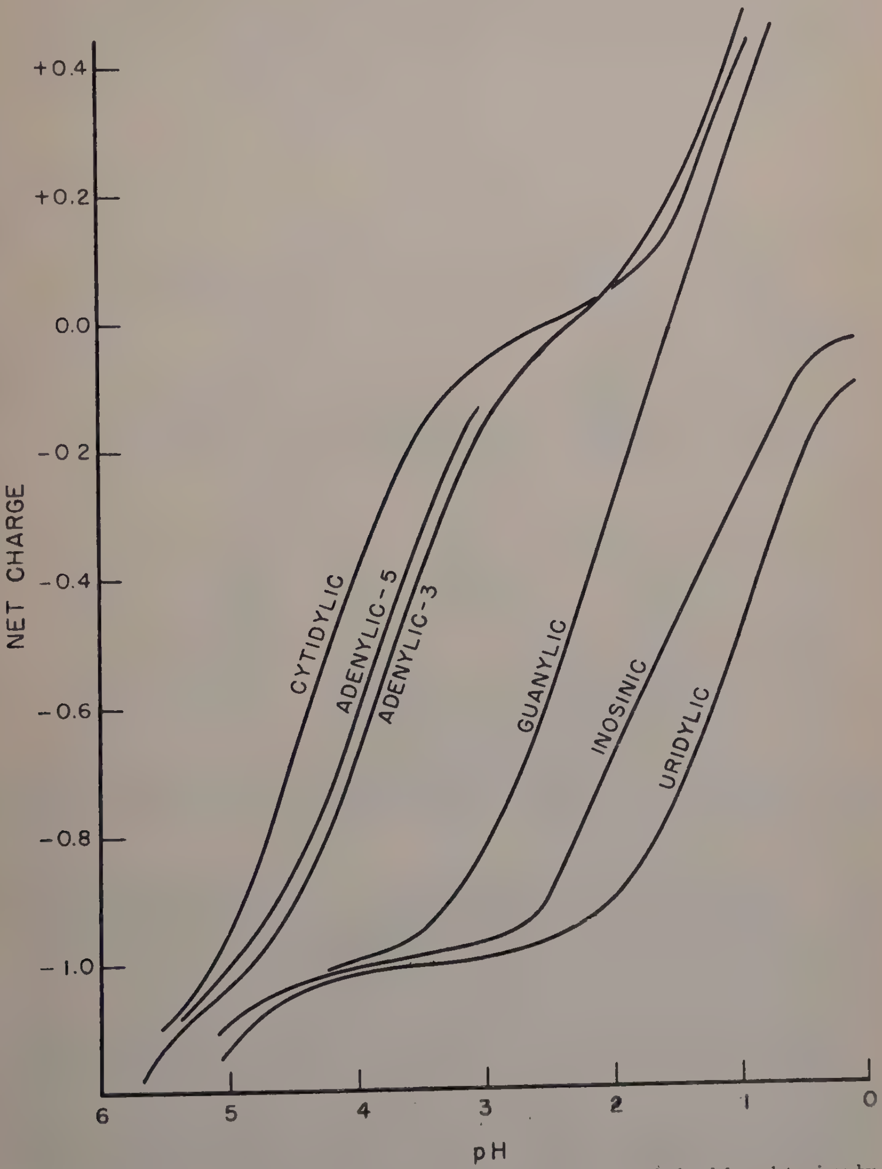

FIGURE 5. Net charge per molecule of ribonucleotide as a function of pH (calculated from data given by Levene) Cohn.[31]

been supposed that the known substances were 3′ phosphates, this led to the early belief that the new *a* substances were the 2′ isomers whose existence had long been made necessary by the hypothesis of a 2′ -3′ internucleotide linkage. The fact that these isomers are interconvertible in acid invalidated the earlier evidence for this correspondence but ion exchange degradation and analysis[35] subsequently served to re-establish it.

Enzymic degradation of ribonucleic acid or of desoxyribonucleic acid with diesterases has more recently been shown, again by ion-exchange analysis, to yield primarily the 5′ nucleotides and not the forms mentioned, see FIGURE 6 (a). The 5′ nucleotides are separable from the *a* and *b* isomers as shown in FIGURE 7 for the adenylic acids.[24, 25] The pK values of the phosphate and amino groups vary significantly from each isomer to the next in the order in which they appear and are undoubtedly responsible for the degree of separation noted.

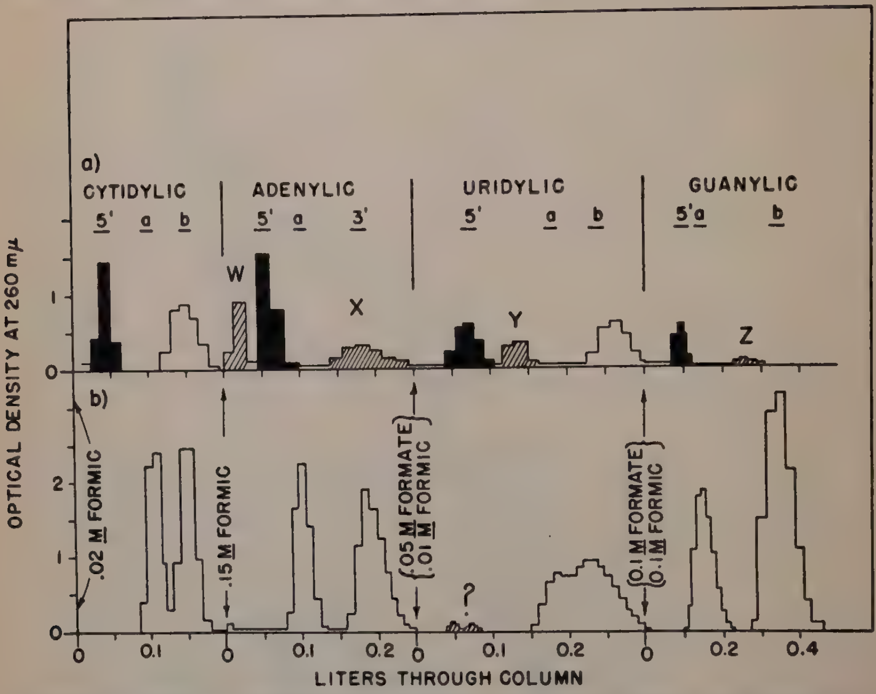

FIGURE 6. Analyses of (a) enzymatic digest, (b) alkaline digest of 20 mg. calf liver ribonucleic acid by ion exchange. (Cohn and Volkin.[34]) Exchanger: Dowex-1-formate, 200–400 mesh 7 cm^2. x 0.88 cm. Influent: formic acid and sodium formate, as shown, 0.6 ml/min.

Doubly phosphorylated nucleotides, such as the pyrimidine diphosphates found in diesterase digests of ribonucleic acid[36] and the adenosine polyphosphates involved in muscle contraction, are more strongly absorbed to anion exchangers, as might be predicted from the increased number of acid groups, and require stronger reagents for their removal. Thus, the pyrimidine diphosphates in the diesterase digests are easily separable from the monophosphates (FIGURE 8), and it has been shown that an easy and exact separation of the adenosine polyphosphates from each other and from muscle adenylic acid is possible (FIGURE 9).

Until recently, polynucleotides had not been chromatographed successfully by ion exchange, although a chromatographic purification of diphosphopyridine nucleotide had been reported by Neilands and Akeson.[38] The mixtures of polynucleotides which were thought to occur in digests of nucleic acids by the appropriate nucleases neither absorbed nor desorbed[39] in a manner such as to permit the precise chromatography which had been demonstrated for the mononucleotides. The shapes of the elution curves indicated that failure to achieve equilibrium between solute and exchanger, a necessary condition for well-defined peaks, was responsible. The same phenomenon had been noted in the case of the polypeptides containing four or more amino acid residues.[40] In the case of the nucleotides, as with the amino acids,[40] it was anticipated and then shown that an ion exchange resin of lower degree of crosslinking, (hence permitting more rapid diffusion of large molecules to the interior), would make possible ion exchange chromatography of substances of higher molecular weight. FIGURE 10 illustrates[41] the separation of a ribonuclease digest of yeast RNA. The various peaks are labeled with the names of the polynucleotides which form their only or their major constituents, using the initials of the respective mononucleotides to represent them. Thus, AC indicates a

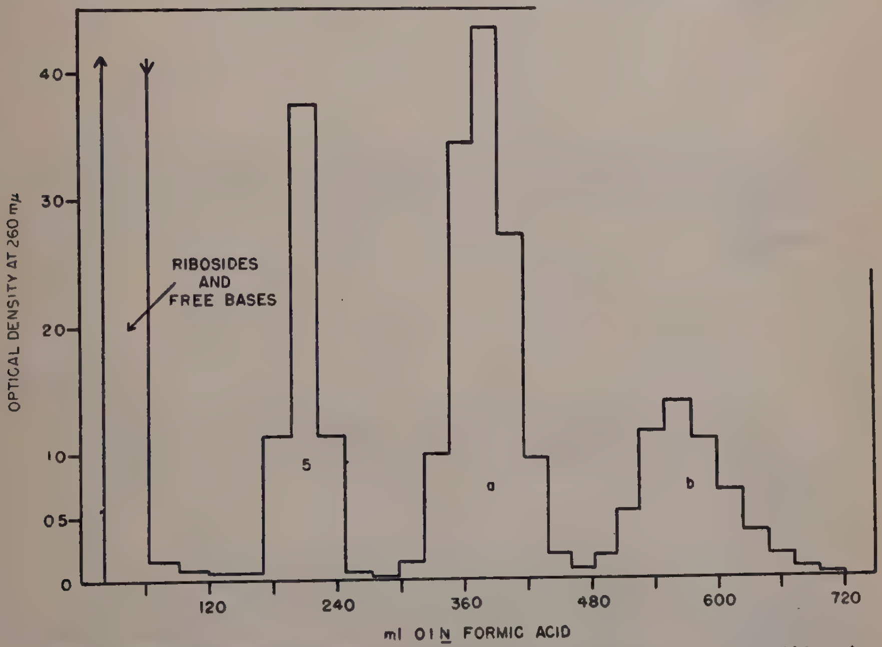

FIGURE 7. Separation of adenosine-5-phosphate and the two adenylic acids of yeast nucleic acid by anion exchange. Exchanger: Dowex-1, 250–500 mesh, 14.0 cm. x 0.74 $cm.^2$ Eluting agent: 0.1 *M*—Formic acid (pH 2.4), 0.5 ml/min. Test material: commercial adenylic acids ~ 20 mg. total.

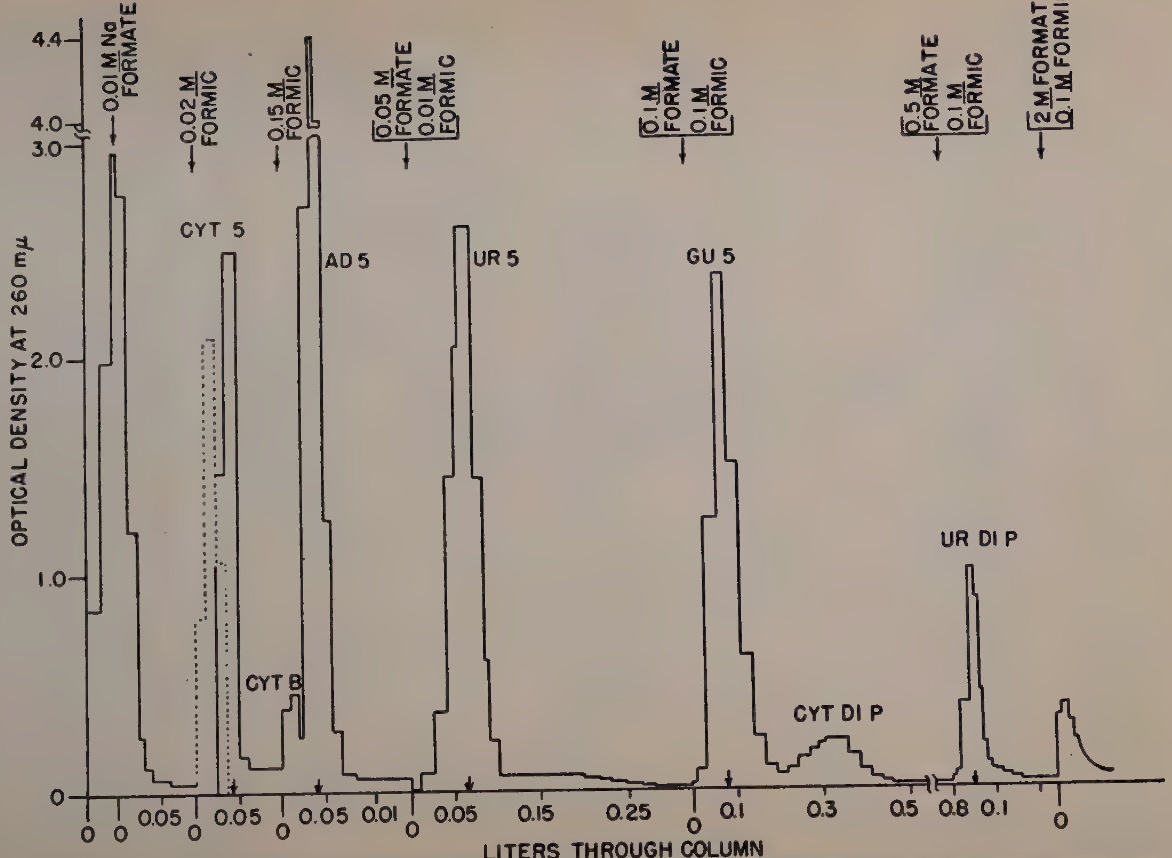

FIGURE 8. Ion exchange analysis of yeast ribonucleic acid digest with purified diesterase from snake venom. Column of Dowex-1, *circa* 400 mesh 5.5 cm. x 0.9 cm.^{2} formate form. Flow rate, 0.8 ml/min. About 24 mg. RNA digest in 0.1 *M* NH_4OH absorbed (from Cohn and Volkin[36]).

dinucleotide of adenylic acid and cytidylic acid in which the cytidylic acid has the free phosphate group; the other phosphate links the two ribose portions and therefore has only one free acid group. It will be noted that the two pairs of isomers, GAC and AGC and GAU and AGU, are separable. Besides indicating the substances that comprise this digest, ion exchange can also produce these substances in quantities suitable for further study. Since at least two groups of tetranucleotides have been found, molecular weights up to 1200 seem to be amenable to conventional ion exchange chromatography, with increased acid or salt to compensate for the increased number of acid groups.

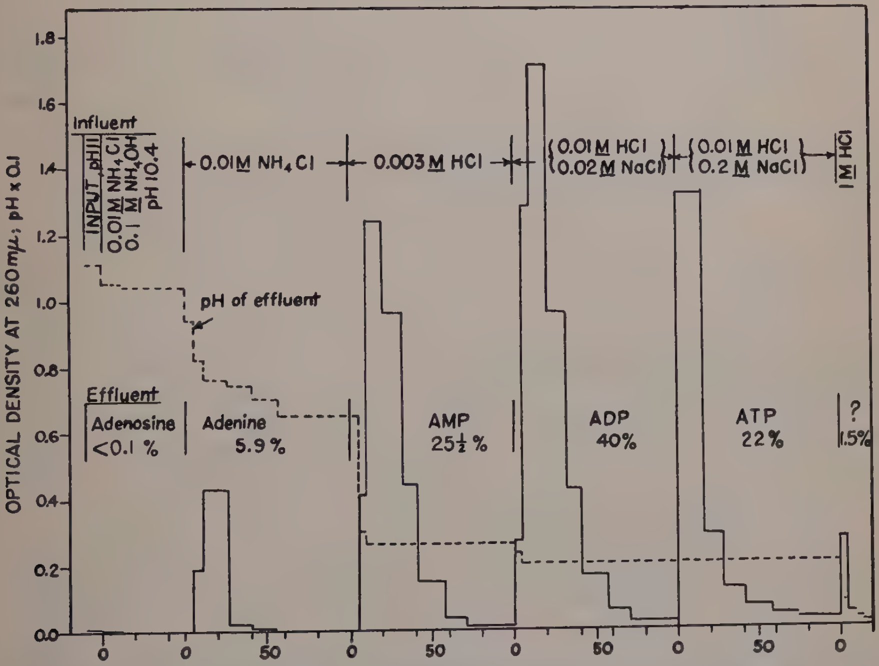

Figure 9. Ion exchange separation of adenosine phosphates (Cohn and Carter[37]). Exchanger: Dowex-1-chloride, 200–400 mesh, 1 cm. x 1 cm.[2] Test material: commercial H_4 ATP. Rate: about 3 ml/min. Abscissa: ml. through column.

Sugars

Sugars are very weak electrolytes and have little tendency, in aqueous solution, to react with the salt form of ion exchangers. Although it had been known for many years that certain polyhydroxy compounds react with borate ion to form complexes which can ionize as acids, it was not until very recently that such complexes were considered[42] for use in ion exchange chromatography. In such a situation, differences in solute-exchanger affinities may be expected to involve differences in the sugar-borate dissociation constants, which depend in turn on borate concentration and pH, in addition to the usual differences of charge of the complex and non-ionic (van der Waal's) attraction. These dissociation constants, in turn, are markedly influenced by the structures of the

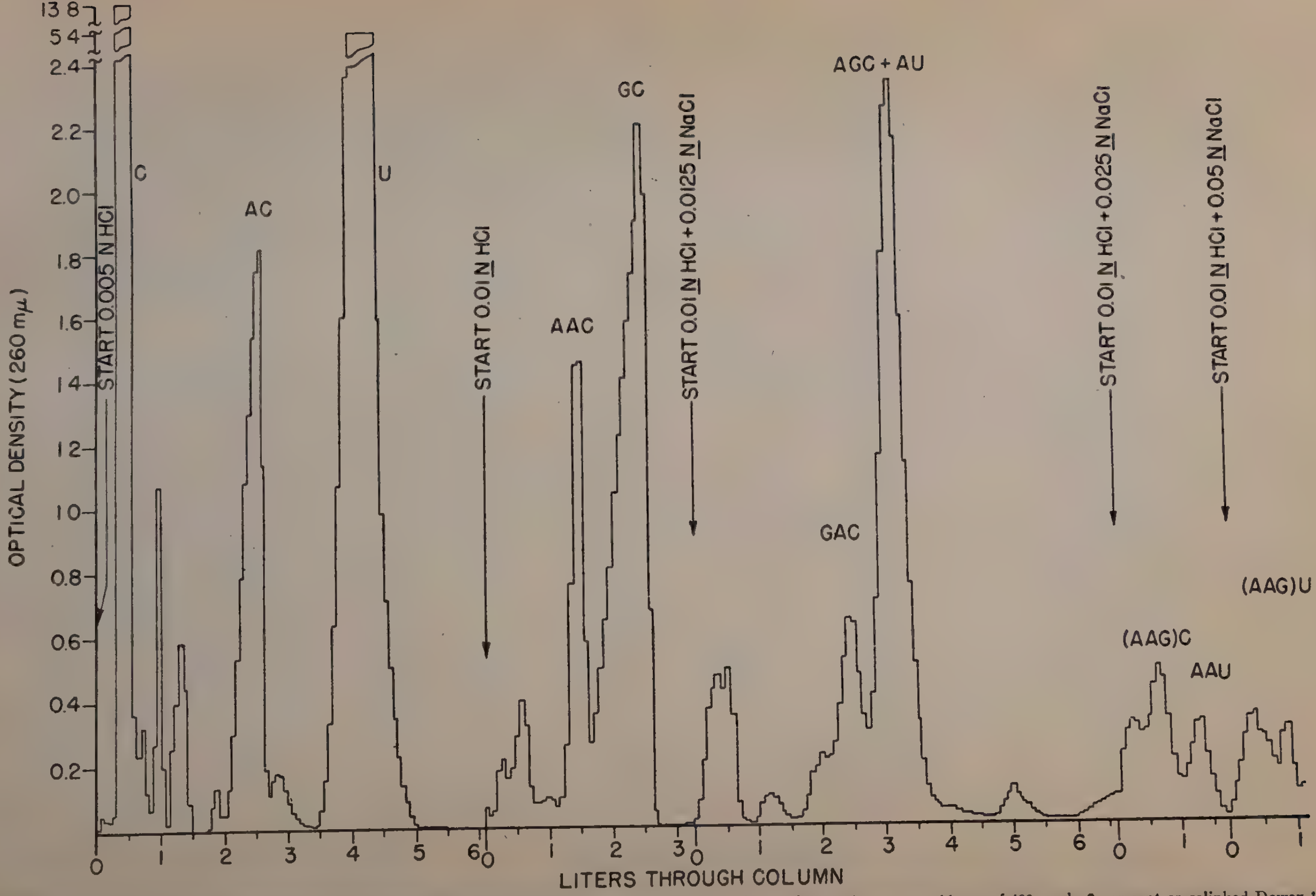

FIGURE 10. Separation of acid-soluble components of ribonuclease-digested yeast ribonucleic acid on column 3.7 sq. cm. x 16 cm. of 400 mesh, 2 per cent crosslinked Dowex-1 Resin. Flow rate 80 ml/hr. 700 mg. of RNA used (Cohn, Volkin, and Doherty[41]).

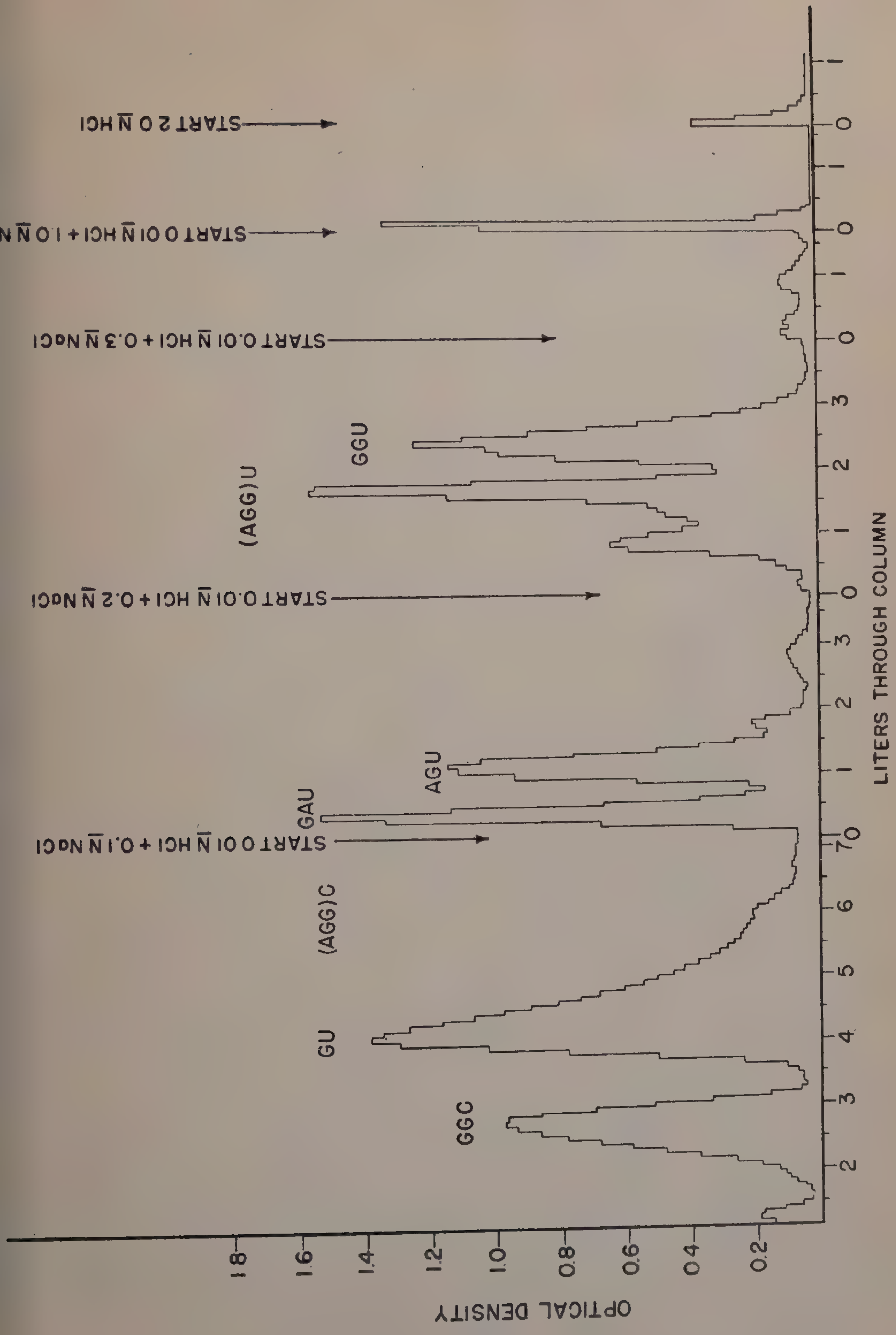

FIGURE 10 (continued)

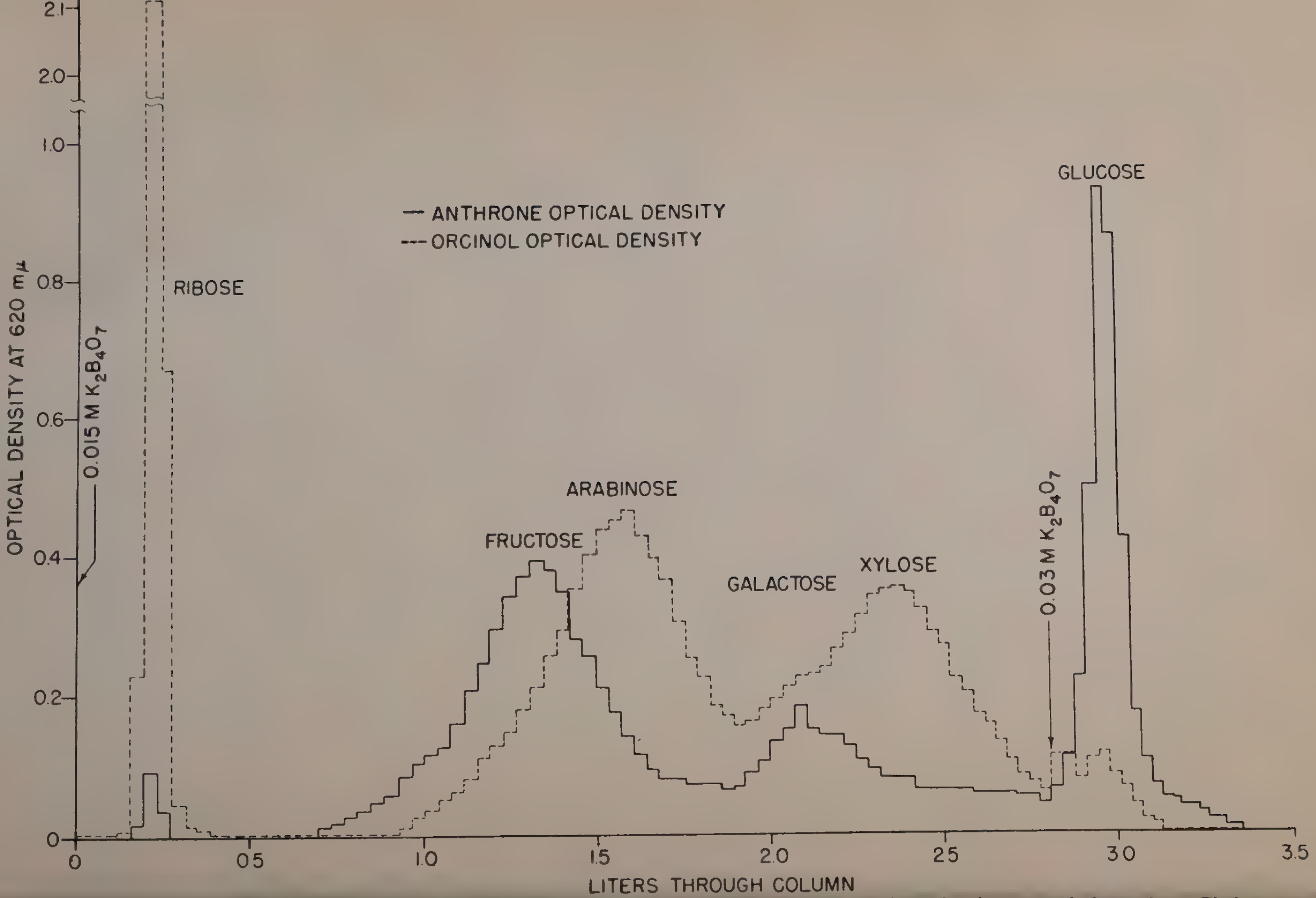

FIGURE 11. Separation of sugars by ion exchange (Khym and Zill[42]). Exchanger: 0.84 sq. cm. x 11 cm. strong base anion resin, *circa* 300 mesh, borate form. Eluting agent:

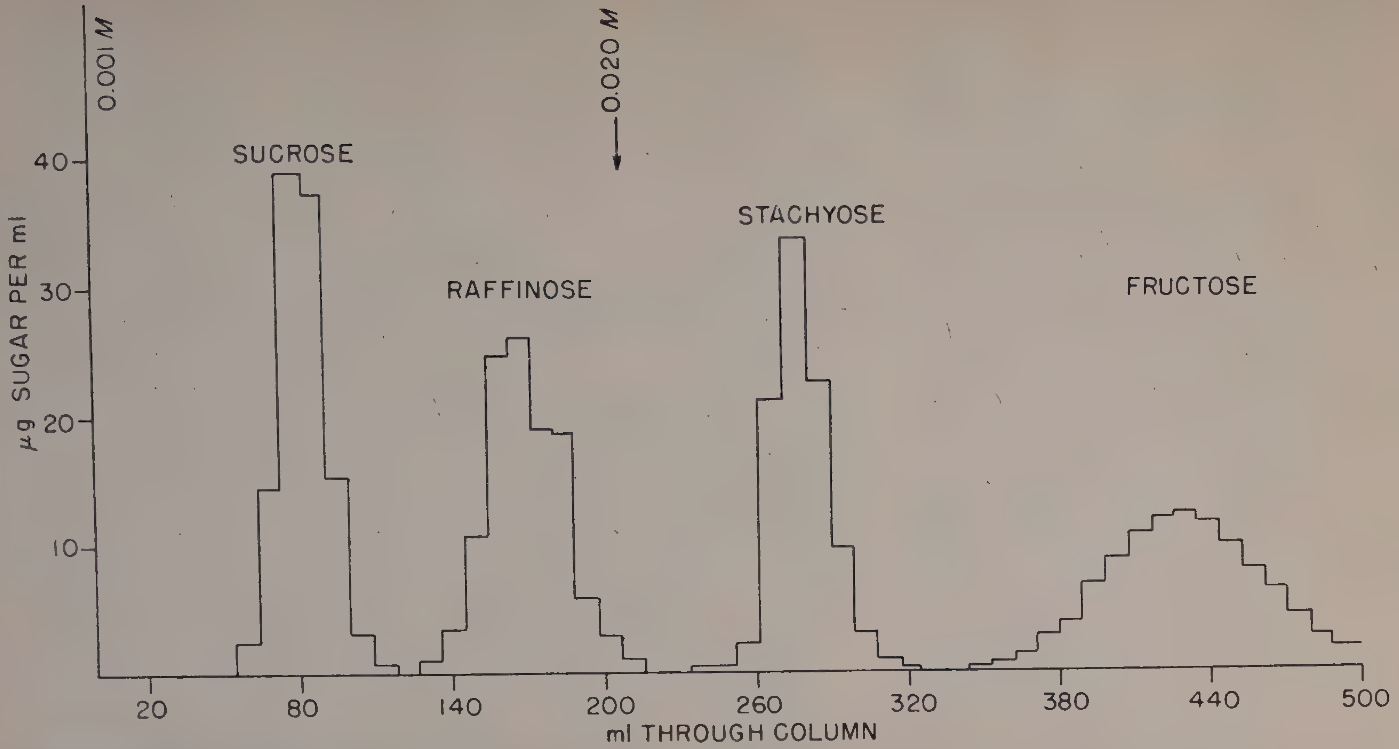

FIGURE 12. Elution of stachyose, a tetrasaccharide, and its potential tri-, di-, and monosaccharide hydrolysis products (1 mg. of each) from Dowex-1-borate, 0.85 cm.^{2} x 2.5 cm., with borate at 1 ml/min. (Noggle and Zill[43]).

sugars involved. Thus, adjacent *cis*-hydroxyl groups form strong complexes, while *cis*-hydroxyls once removed form weaker ones. *Cis*-hydroxyls formed by the opening of a furanose or pyranose ring will be able to form complexes to the extent that the ring opens; and enolization of ketone groups will influence the number of reacting groups; as will mutarotation and furanose-pyranose interconversion, because of the greater degree of complexing of furanose structures.

Two examples of the power of this approach are shown. The separation of a mixture of pentoses and hexoses is shown in FIGURE 11. Although mixed peaks are obtained, the isolation of each hexose from the other hexoses and of each pentose from the other pentoses is essentially complete.

The separation of a tetrasaccharide from a constituent trisaccharide, a constituent disaccharide, and a constituent monosaccharide, such as might be found in a partial hydrolysate of the parent molecule, is shown in FIGURE 12.

It is possible to recover each component as a pure sugar, free of borate, which makes the method suitable not only for analysis but for the preparation of pure sugars. It thus constitutes a new tool in sugar chemistry and biochemistry.

Sugar Phosphates, Organic Acids, and Alcohols

The phosphorylated intermediates involved in glycolysis constitute a mixture which is difficult to resolve by classical methods. The discussion of the separation of the nucleotides makes it clear that diphosphates may be separated from those substances containing one or no phosphate groups, but the separation of the sugar monophosphates (fructose-6, glucose-1, glucose-6, and ribose-5) cannot be accomplished[44] by chloride ions alone at any pH.

The ease of separation of the neutral sugars by borate complex formation indicated that borate complexing might be useful in this situation. The analysis of a complex mixture is shown in FIGURE 13. In this analysis, the substances amenable to separation as borate complexes are removed first, with chloride exchange at lower pH values being responsible for the remainder.

The uronic acids are difficult of analysis in biological materials, owing to the interference caused by sugars. The neutral sugars tend to pass through ion exchange columns in the absence of borate; the chromatographic separation of the retained uronic acids can then be accomplished as shown in FIGURE 14. Mannuronic acid appears in the same fractions as the glucuronic acid. The sugars which break through can, of course, be absorbed on a borate column and analyzed separately.

The sugar alcohols—sorbitol, dulcitol, and mannitol—are separable in exactly the same way as the neutral sugars.[46]

Finally, the mixture of carboxylic acids encountered in the citric acid cycle may be considered. In the absence of phosphates and without the use of complexing agents, variation in ionic and organic properties and in the number of acid groups can be exploited for the separation of many of these substances. As is shown in FIGURE 15, simple replacement by formate ion makes it possible to recover many of the substances in pure form, although a few which occur as mixtures must be separated in subsequent manipulations.

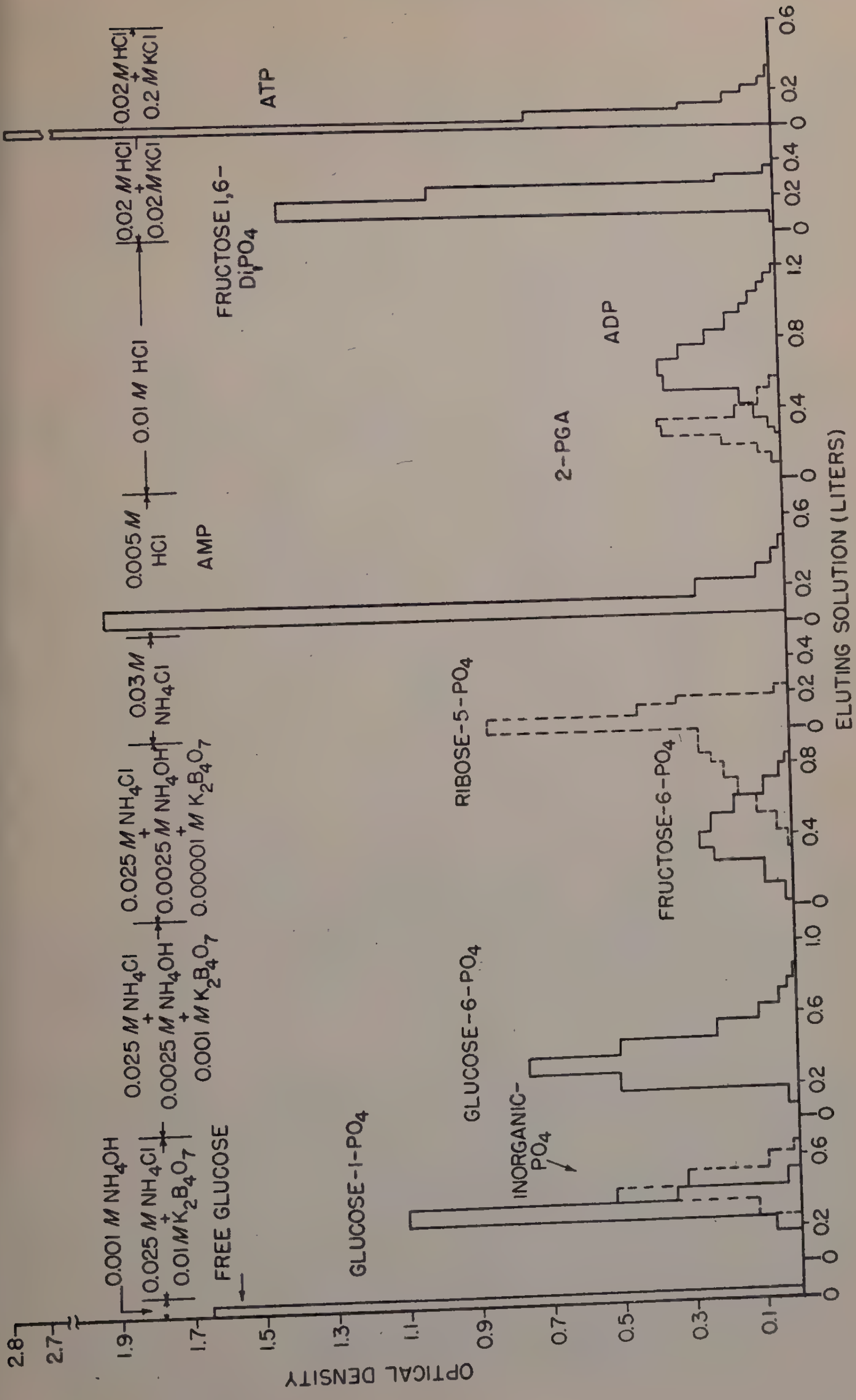
OPTICAL DENSITY
2.8
2.7
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
0.001 M NH4OH
0.025 M NH4Cl + 0.01 M K2B4O7
0.025 M NH4Cl + 0.0025 M NH4OH + 0.001 M K2B4O7
0.025 M NH4Cl + 0.0025 M NH4OH + 0.00001 M K2B4O7
0.03 M NH4Cl
0.005 M HCl
0.01 M HCl
0.02 M HCl + 0.02 M KCl
0.02 M HCl + 0.2 M KCl
FREE GLUCOSE
GLUCOSE-1-PO4
INORGANIC-PO4
GLUCOSE-6-PO4
FRUCTOSE-6-PO4
RIBOSE-5-PO4
AMP
2-PGA
ADP
FRUCTOSE 1,6-DiPO4
ATP
0
0.2
0.6
1.0
0.4
0.8
1.2
ELUTING SOLUTION (LITERS)

Figure 13.

The separation shown in FIGURE 15 is of additional interest in that it demonstrates the use of a continually changing solvent rather than one which is changed in a series of discrete steps. This separating principle, of which this is perhaps the earliest published example, avoids sudden changes in the bed volume (this change is larger, the lower the degree of crosslinking in the resin) and makes each peak sharper.

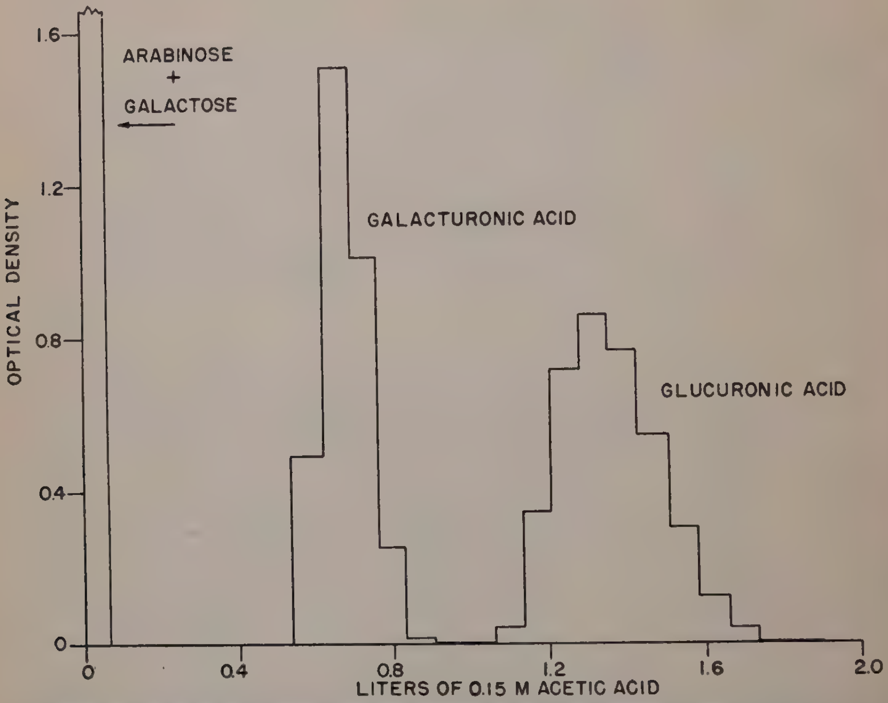

FIGURE 14. The separation of galacturonic and glucuronic acids in the presence of sugar. Exchanger: 0.85 sq. cm. x 12 cm. Dowex-1, *circa* 300 mesh, acetate form. Eluting agent: 0.15 M Acetic acid at ~ 2.5 ml/min. Test material: 5.0 mg. each of arabinose and galactose, 10 mg. each of galacturonic and glucuronic acids in 10 ml. of 0.02 M sodium hydroxide (Galactose was determined by anthrone method at 620 mμ, the other materials by orcinol method at 660 mμ.).

Proteins

In the consideration of the ion exchange chromatography of the amino acids, nucleotides, sugar derivatives, and carboxylic acids, it has been possible to indicate, with some degree of assurance, the roles played by ionic and nonionic forces with respect to the molecules in question. With proteins, lack of detailed knowledge of chemical structure precludes any such discussion and should, perhaps, exclude these substances from consideration in this review. Nevertheless, the practical importance of the column chromatography of proteins demands at least a mention of the achievements to date.

It has been shown that some protein solutions can be deionized, as in dialysis, by passage through ion exchange beds without harm to the protein. Thus, Reid and Jones[48] were able to desalt plasma protein solutions and to achieve fractionations of the proteins without denaturation. Gilbert and Swallow[49] observed partial to complete inactivation of urease and α- and β-amylase in passing through an acid cation exchanger, while pepsin was not inactivated. The use of a weak-base anion exchanger to remove acids from vasopressin preparations has been reported.[50] Many similar experiments, not involving absorption and desorption of protein, have been performed in various laboratories.[51]

More recently, several instances of the adsorption and elution of protein, undenatured and with hormonal or enzymic activities intact, have been reported. Among these are cytochrome c,[52] ribonuclease,[53] lysozyme,[54] bacterial pectinase,[55] and the adrenotropic hormone.[56] In some of these cases the material has been readsorbed and reeluted without deviation from the original chromatogram, a further proof of the integrity of the desorbed molecule.

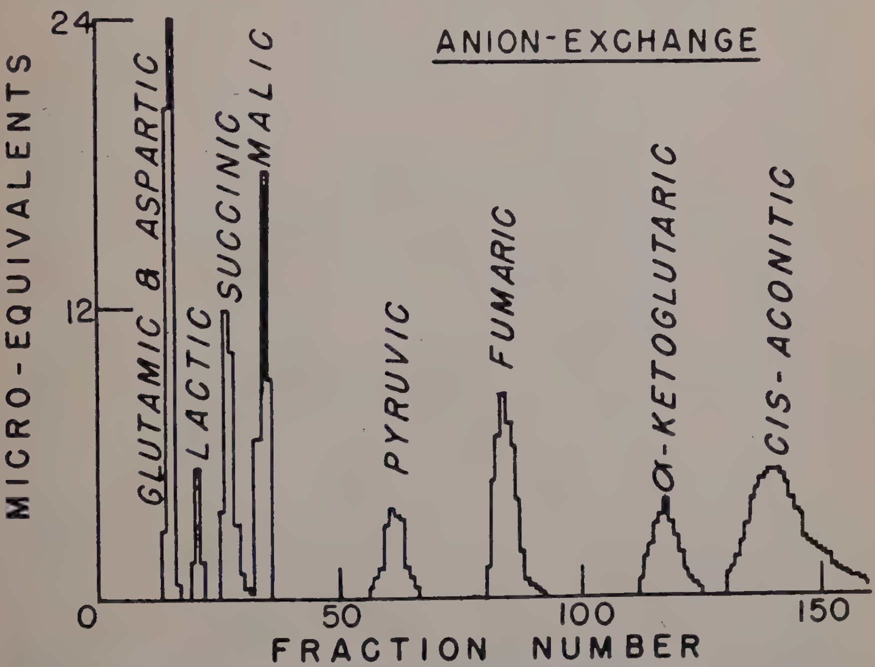

Figure 15. Separation of organic acids by anion exchange. Elution at 40 drops/min. with formic acid gradually increasing in concentration from 0.1 to 6 N. Dowex-1-formate column 0.8 cm.^{2} x 11.5 cm. Fractions = 40 drops each. Malonic, citric, and isocritic acids appear with pyruvic acid. (Busch, Hurlbert, and Potter.[47])

All these later cases have utilized the carboxylic acid cation exchanger, Amberlite IRC-50. It is generally assumed that deleterious exposure to acid and to the strong electrical fields of a strong-acid exchanger are avoided in this fashion. In the absence of a determinaton of the mechanism at work and the rates of destruction, if any, however, it is well to reserve judgment as to the possible disadvantages attending the use of other exchangers.

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PURIFICATION OF ANTIBODIES BY MEANS OF ANTIGENS LINKED TO ION EXCHANGE RESINS*

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Methods have been developed for the purification of isohemagglutinins. They also appear to be adequate for the resolution of the antibodies occurring in the γ-globulin fraction of pooled human plasma.

Previously, physical chemical methods[1] have led to a preferential concentration of antibodies in different subfractions. Isoagglutinins, for instance, have been concentrated 16 times[2] by fractional precipitation with ethanol at specified pH and ionic strength. The degree of purity of the final preparations, however, has remained less than 1 per cent. Among physical methods, electrophoresis convection appears to be the most powerful tool for the subfractionation of γ-globulin.[3] Although a partial fractionation has been achieved, the physical chemical properties of antibodies are so similar that thus far no pure compound has been isolated solely on this basis.

In an attempt to obtain specific antibodies of high purity, we have made use of the most specific precipitating agent, namely, the antigen to the corresponding antibody. Similar methods have been employed previously. They have 4 steps in common: (1) combination of the antibody with its antigen; (2) purification of the complex by removal of occluded or combined impurities; (3) dissociation of antibody from the specific precipitate or "agglutinate"; (4) separation of the released antibody from the solution.

Whereas the first two steps have been effected with ease, methods of reversing serological reactions (step 3) have not been satisfactory. Antibodies to carbohydrates have been extracted in small yield from specific precipitates at high concentrations of sodium chloride.[4] In the system egg albumin-anti-egg albumin dilute acid or base has been used.[5] Kabat[6] has shown that in certain systems the dissociation of specific precipitates by barium hydroxide and barium chloride affects the physical chemical properties of the antibody. Campbell and his coworkers have demonstrated that the yield of antibodies to artificial antigens can be increased by combining acid elution with hapten dissociation.[7]

The separation of the released antibody from the antigen (step 4) can be effected by centrifugation, when sufficient antibody remains in the precipitate to maintain the antigen insoluble. Haurowitz and his coworkers[8] have adsorbed antibody on a dye-antigen. The latter was insoluble at acid pH, at which the dissociation of antibody took place. Sternberger and Pressman[9] have devised a procedure to render antigens insoluble at alkaline pH. Campbell, Luescher and Lerman[10] have prepared an insoluble antigen by coupling bovine albumin to a derivative of cellulose. For further details on specific

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* The work reported here was supported by funds of Harvard University, grants from foundations, the National Institutes of Health, and by contributions from industry.

and non-specific methods of purifying antibodies, the reader is referred to previous reviews.[11]

Methods of maintaining antigens insoluble—thus rendering possible columnar operations—by coupling antigens to ion exchange resins, are first considered in this communication. Methods are then described for recovering human antibodies in good yield from resin-antigen complexes avoiding changes in pH known to affect the physical chemical properties of the antibody.

I. Preparation of Insoluble Antigens

A. Cellular antigens. An antigen present as a constituent of an insoluble, stable particle, may sometimes be used for the adsorption of the antibody. Bacteria or red blood cells, however, though formed elements, must be modified because of their fragility. In the case of the ABO blood group system the removal of the antibody from plasma is of practical importance in itself. The views concerning the safety of transfusing human plasma containing isoagglutinins have changed during the past few years, since plasma with a high concentration of anti-A or anti-B isoagglutinins has been known to produce serious transfusion reactions. Isoagglutinins have been neutralized by addition of specific blood group substance from animal sources. In this procedure a heterologous antigen is introduced and the antibody cannot be recovered.

The blood group substance of red blood cells, discarded in large amounts after blood has become outdated, has been our starting material. These cells are not useful as antibody adsorbents because of their fragility. The cells have been modified in order to insure: (a) their insolubility; (b) their capacity for isoagglutinins; (c) their avidity, which is considered to depend upon the rate of antibody adsorption; (d) the physical coarseness of the antigen preparation in order to achieve high flow rates in columnar operations.

The effect of heat on red blood cells in producing a stable insoluble material was first investigated. Boiling of washed erythrocytes[12] for a short time, followed by drying them from the frozen state, consistently diminished the cell avidity for isoagglutinins but only slightly affected the capacity of the cell. Following adsorption, traces of methemalbumin were found in the plasma as shown by an adsorption maximum at 405 mμ. Prolonged boiling, followed by drying of the denatured cells at 110°C, prevented the extraction of heme compounds by the albumin of plasma. The avidity and the capacity of the cells, however, were decreased to less than half the original value.

The blood group antigen is a polysaccharide located in the stroma of the red blood cell. Stroma has been prepared by laking cells with distilled water at pH 6 and repeated washings. Stickiness and a tendency to form colloidal suspensions at ionic strengths greater than 0.1 at first rendered the stroma difficult to process. The properties of stroma were improved by incubation with 0.5 per cent formaldehyde at 37°C for 12 hours. This treatment was found to be superior to the action of other tanning agents, among them glyoxal and tannic acid. An alternative procedure consisted in incubating washed cells with trypsin for 10 minutes at 37°C. After lysis and washing, a nearly colorless, rapidly settling stroma was obtained. Its avidity was increased as compared with untrypsinized stroma. A gram of this material was found sufficient, in a batch operation, to reduce the isoagglutinin titer in 100 ml. of plasma from 64 to 2. The stroma-isoagglutinin complex was separated from plasma by centrifugation.

Stroma modified in this way still had one disadvantage when used in columnar operations: the membranes were pressed together and caused plugging of the column. Inert particles were therefore coated with the antigen. Among the large number of substances tested, synthetic exchange resins of several types appeared satisfactory in maintaining antigens in the solid state. Anion exchange resins were found superior to cation exchange resins in that they exerted a stronger affinity for stroma. This effect was marked when the stroma had previously been treated with formalin. It has been assumed that this resulted from an effect upon the net charge of the stroma, presumably increased in the presence of formaldehyde. As a result binding by positive groups of the anion exchange resin was induced.

The procedure that has been developed for preparing stroma bound to resin was based on the above observations. Stroma from 250 ml. packed red blood cells (2 gms. dry weight) was dispersed in 250 ml. 0.05 per cent sodium tetraborate, and formaldehyde was added to give a final concentration of 0.1 per cent. 80 gms. of a dried anion exchange resin* were stirred vigorously with the suspension until all liquid was adsorbed by the resin. A thin film of stroma remained on the surface and in the cracks of the beads. The resin, so prepared, was washed, dried and could be stored without loss of activity. Due to the tanning action of the formaldehyde, the stroma-resin was insoluble at an ionic strength of 0.15. The concentration of formaldehyde should not exceed 0.3 per cent during the preparation of the resin. FIGURE 1 represents the adsorption of anti-A isoagglutinin by different stroma preparations. Each preparation was derived from 10 ml. packed type A red blood cells. The adsorption capacity and the avidity of the stroma-anion exchange resin was essentially unchanged from that of untreated stroma. Formaldehyde (0.5 per cent) did not affect the final capacity of the stroma for isoagglutinins whereas the avidity was decreased. Non-specific adsorption, as demonstrated by a control experiment with type B stroma, was negligible. In addition to its tanning action, formaldehyde also functioned as an antimicrobial agent. Where formaldehyde was not used the antigenic material could be sterilized by cathode rays at 0°C without loss of activity.

Hyperimmune plasma was freed in this manner from its agglutinins by passage through a stroma-resin column. The flow rates were of the order of 5–10 ml. per minute when using a column 4 inches in height and 1.5 inches in diameter. By modifying the procedure the highly sensitive Rh antigen from Rh positive cells has been combined to resin at neutral pH, and used for the adsorption of both complete and incomplete antibodies.

Resin treated in this way served as a supporting material for stroma and has rendered possible the adsorption of agglutinins as a columnar operation. Similar procedures can be employed with other antigens combined with insoluble particles.

B. General procedure for the preparation of antigen-resins. In systems where the antigen was not part of a cell but a soluble substance, the procedure was modified in order that the antigen could not be displaced from the resin column by a solution containing the antibody. Soluble as well as cellular antigens were linked, therefore, to resins by a chemical bond. This procedure was possible only if the groups involved in the antigen-antibody reaction were unaffected or but slightly affected by the linking of the antigen to the resin.

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* XE 75 chloride form supplied by Rohm and Haas Company.

The active groups of a cation exchange resin (XE 64 or XE 93)* were converted to acid chlorides by refluxing the dry resin with thionyl chloride. After the excess of reagent was removed by distillation, it was found that 92 per cent of the carboxyl groups of the resin XE 64 had been converted.

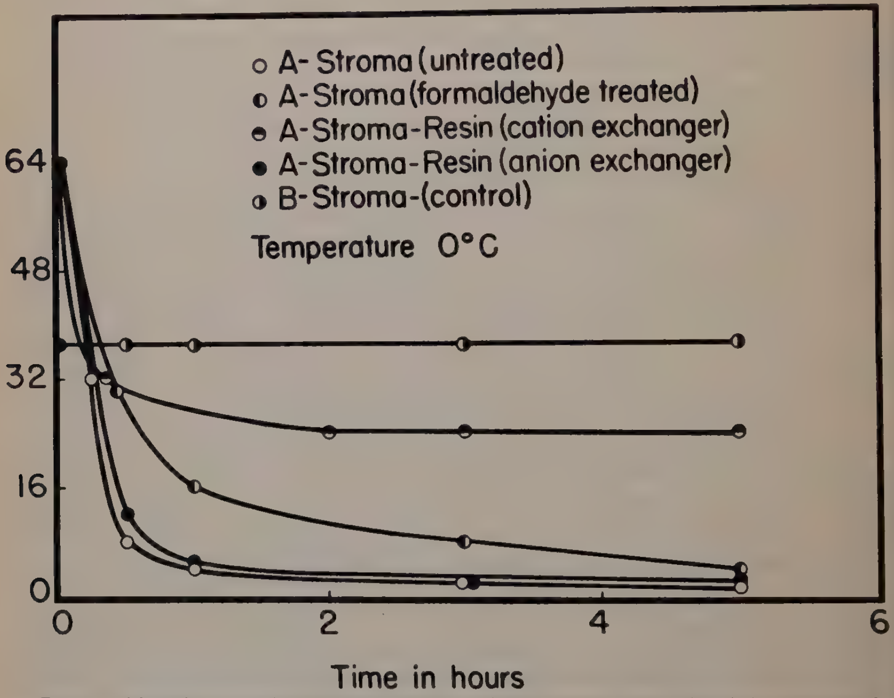

FIGURE 1. Adsorption of anti-A isoagglutinin from 50 ml. plasma by stroma preparations derived from 10 ml. packed type A cells.

Blood group antigens. These were linked to carboxylated resin converted to its acid chloride by adding to it a fine suspension of stroma in 1 N potassium bicarbonate. A decrease in blood group activity in the suspension, separated from the beads by centrifugation, could be accounted for by a corresponding increase in activity of the beads which persisted after thorough washing. A solution of 2 N ammonium acetate, adjusted to pH 8 by the addition of potassium bicarbonate, was found to be the most effective reagent for hydrolyzing the excess acid chloride from the resin without damage to the antigen. The rate of antibody adsorption of the stroma combined in this manner to a cation exchange resin at first appeared not to have been affected. The final capacity was, however, considerably reduced as compared with untreated stroma. A similar loss of activity was observed in the case of human serum albumin and horse anti-serum albumin. The causes of this behavior were more readily investigated in this system.

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* Supplied by Rohm and Haas Company.

Human serum albumin-resin. Carboxylated resins were converted to their chlorides by refluxing the resin, XE 64, for different lengths of time with various dilutions of thionyl chloride in chloroform. The resin was washed with an excess of chloroform and dried *in vacuo*. The degree of substitution was calculated from the total number of carboxyl groups originally present on the resin and the number of chloride groups which could be titrated after a sample had been hydrolyzed by 2N sodium hydroxide. Albumin was linked to the resin under the same conditions as were used with stroma. The binding of albumin was determined by measuring the optical density in the supernatant solution, corrections being made for the removal of water by the resin. The adsorption of horse anti-serum albumin antibody by three different resins is reported in TABLE 1. It appeared from those data that the reagents used for the preparation of the antigen-resin only partially affected the specificity of the serological reaction. The non-specific adsorption of antibody on an untreated resin (XE 64) was small, and the antibody could be washed off with saline.

TABLE 1

ADSORPTION OF HORSE ANTI-SERUM ALBUMIN ANTIBODY BY DIFFERENT ALBUMIN RESINS
(XE 64: carboxylated resin partly converted to acid chloride)

Resin XE 64 No.	% of COOH converted to COCl	mg. human serum albumin fixed per gm. of dry resin	mg. anti-serum albumin* before adsorption	mg. anti-serum albumin* after adsorption	mg. anti-serum albumin adsorbed on resin
1	0	6†	47	43	4
2	92	96	47	24	23
3	11	71	47	8	39

* Determined according to the method of Gitlin.[13]
† Albumin can be washed off with 0.15 M saline.

It is noteworthy that the resin binding 71 mg. of albumin adsorbed more antibody than the resin binding 96 mg. of albumin. This finding suggested that the larger the number of groups of the antigen that are linked to the resin, the smaller will be the number of groups available for combination with antibody. A factor which might account for this is the steric arrangement of the antigen on the resin, as demonstrated in FIGURE 2. Denaturation by the higher amount of hydrochloric acid released during the coupling of albumin to resin No. 2 was minimized by continuously adjusting the pH with vigorous stirring at 1°C by addition of borate buffer. The destruction of remaining acid chloride was effected by ammonium acetate as described in the case of stroma.

Influenza virus-resin. Purified influenza virus has been obtained by making use of its hemagglutinating properties. Allantoic fluid from chick embryo inoculated with the PR8 strain of influenza A virus was dialysed at pH 7 and passed through a stroma-resin* column prepared as described in part 1 *A*. The adsorptions of the virus by chicken red cells, human red blood cells and by the stroma-resin are compared in FIGURE 3. At 0°C 95 per cent of the virus was adsorbed on the stroma-anion exchange resin within 10 minutes. After washing, the virus could be eluted nearly quantitatively with saline at 37°C. The dissociation had to be carried out within one hour, for at longer times spontaneous elution took place. The average increase in concentration, calculated on the basis of protein-nitrogen, was 20-fold. Control experiments with the same anion exchange resin but without stroma indicated that only a small amount of the virus was adsorbed under the same conditions.[14]

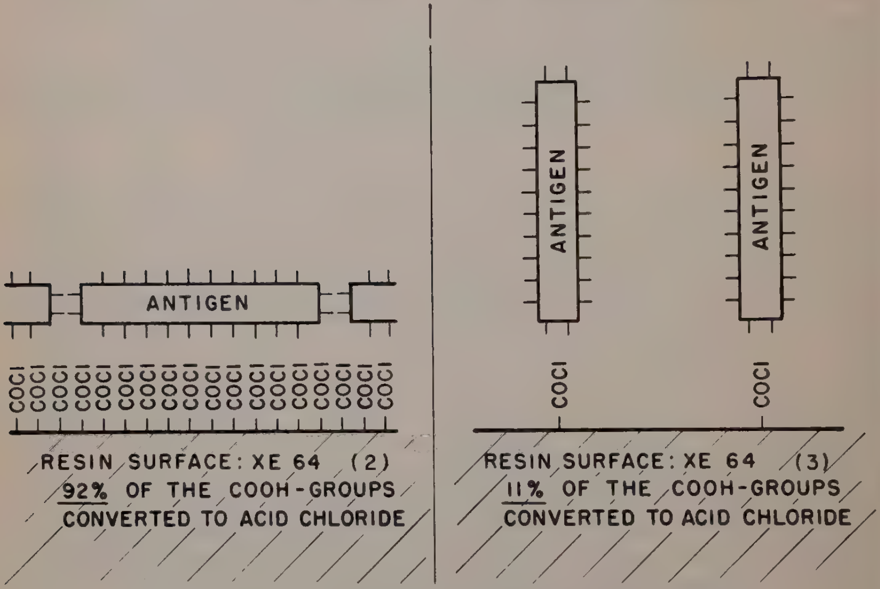

FIGURE 2. Schematic representation of the linkage between resin and antigen.

More recently, influenza virus† has been linked irreversibly to a cation exchange resin by using the acid chloride method described in the preceding section. The adsorption of influenza A antibody on such virus columns is summarized in TABLE 2. The presence of non-specific hemagglutination-inhibitors was excluded by using highly purified γ-globulin as a starting material. It appears from these data that the serological properties of the virus antigen are affected by chemical alteration in a manner similar to the serological properties of albumin as shown in TABLE 1.

An alternative procedure for linking antigens to resins by a diazotization procedure has been employed. As resins with free aromatic amino groups were not available, a sulfonated aromatic amine (*e.g.*, dye) was first adsorbed on an anion exchange resin. The adsorbed amine was then diazotized and coupled to the antigen. The affinity of the dye for the anion exchange resin increased with the number of sulfonic groups introduced into the ring system of the dye. The tightness of the complex, however, could not be accounted for solely on the basis of coulomb forces between the sulfonic groups of the dye and the posi-

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* IRA 410 supplied by Rohm and Haas Company.

† PR8 strain kindly supplied by Dr. I. Gordon (State of New York Department of Health, Albany, N. Y.).

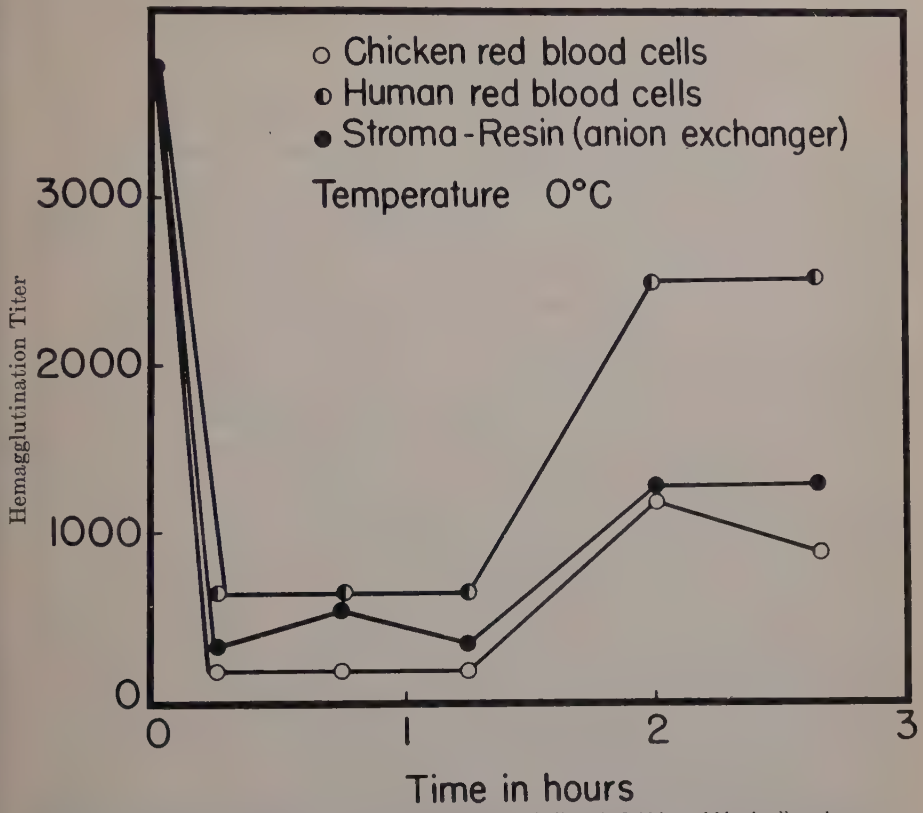

FIGURE 3. Adsorption of influenza A virus from PR 8 infected allantoic fluid by red blood cells and stroma-resin: 0.25 ml. packed chicken red blood cells, 0.25 ml. packed human type O red blood cells, stroma derived from 0.25 ml. human type O red blood cells combined to anion exchange resin.

TABLE 2

FIXATION OF PR8 VIRUS ON RESIN AND ADSORPTION OF ANTI-PR8 FRACTION FROM HUMAN γ-GLOBULIN

Resin XE 64 No.	% of COOH converted to COCl	Hemagglutinin titers of dialyzed infected allantoic fluid		Hemagglutination inhibition titers of human γ-globulin fraction after adsorption	
		Before fixation on resin	After fixation on resin	On control-resin XE 64	On virus-resin XE 64-PR8
1	0	256	256	—	—
2	92	1024	2	(64)	32
3	11	256	32	(128)	16

tive groups on the resin, for neither concentrated acid nor base liberated the dye from a resin with a high degree of crosslinkage. Moreover, dyes of high molecular weight have a greater affinity for resins than compounds with otherwise identical groups of low molecular weight. The number of linkages between resin and antigen was controlled by the amount of amine (or stabilized diazo-compound) adsorbed to the resin. This procedure was more time-consuming than that using the acid chloride technique.

II. Elution of Antibody from Antigen-Resin

In evaluating methods for the dissociation of antigen-antibody complexes, it was noted that: (a) the forces involved in the serological reaction differed from one system to another; as a rule, antibodies to polysaccharide antigens have been more easily dissociated than antibodies to protein antigens; (b) antibodies were released more readily from a heterologous than from a homologous antigen; (c) in many instances the recovery of antibody was increased when the antigen had been chemically altered, a fact bearing on the experiments in which antigens were chemically linked to the resins; (d) regardless of the dissociation method the yield of antibody is dependent on the ratio of antigen to antibody during the adsorption phase. Specific precipitates formed in the presence of antibody excess are easier to dissociate than specific precipitates which are not saturated with antibody, an observation which probably reflects the multivalence of the antigen as well as steric hindrances. When present in excess, antibody molecules appear not so well adapted to the combining site of the antigen as in the region of antigen excess; (e) in order to elute pure antibody, it was necessary in previous methods to disperse specific precipitates during the washing procedure. In the case of antigens combined to resin columns saturated with antibody, saline—5 to 10 times the volume of the original plasma—was passed through the column until the effluent was protein-free; (f) the time allowed to elapse between adsorption and elution should be as short as possible;[15] specific precipitates which had stood overnight were more difficult to dissociate than freshly prepared precipitates.

These preliminary observations suggest that, in comparing different eluting agents, the reaction conditions be maintained constant throughout all operations.

A. Elution of isoagglutinins. The effect of the pH on the release of isoagglutinins from stroma-resin is graphically represented in FIGURE 4. Yields exceeding 50 per cent were obtained at pH values smaller than 2.5 and larger than 11. Although the groups which determine the antibody specificity were extremely stable, the physical chemical properties of isoagglutinins released at pH 3, which still reacted with red blood cells, were affected. On the other hand, using high concentrations of either salt or glycine, the antibody was exposed to milder conditions, but the recovery was small.

In the case of blood group antibodies, a few compounds have been found which appear to release isoagglutinins by destroying antigenic sites on the stroma resin. Among them are potassium periodate at concentrations of 0.1 to 0.5 mM and pH 6.0.[16] Release of smaller amounts of antibody by lysozyme and extracts of *Vibrio cholerae* probably is due to digestion of the blood group polysaccharide on the stroma surface.

Simple sugars which are contained in blood group substance inhibit the agglutination of red blood cells by their corresponding agglutinins.[17] The lowest concentrations of sugars which inhibit agglutination are listed in TABLE 3; N-acetyl-D-glucosamine and D-galactose—the main components of the blood group polysaccharide—are among the strongest inhibitors of agglutination. At 37°C the latter sugars have been found effective in eluting antibody from blood group substance. An elevation of the temperature generally favors the dissociation of specific precipitates, since the antigen-antibody reaction is exothermic. In the case of stroma-isoagglutinin complexes, this effect is small at 37°C. It is large enough, however, for a mixture of galactose and glucosamine or lactose to prevent the recombination of the released antibody with the antigen.

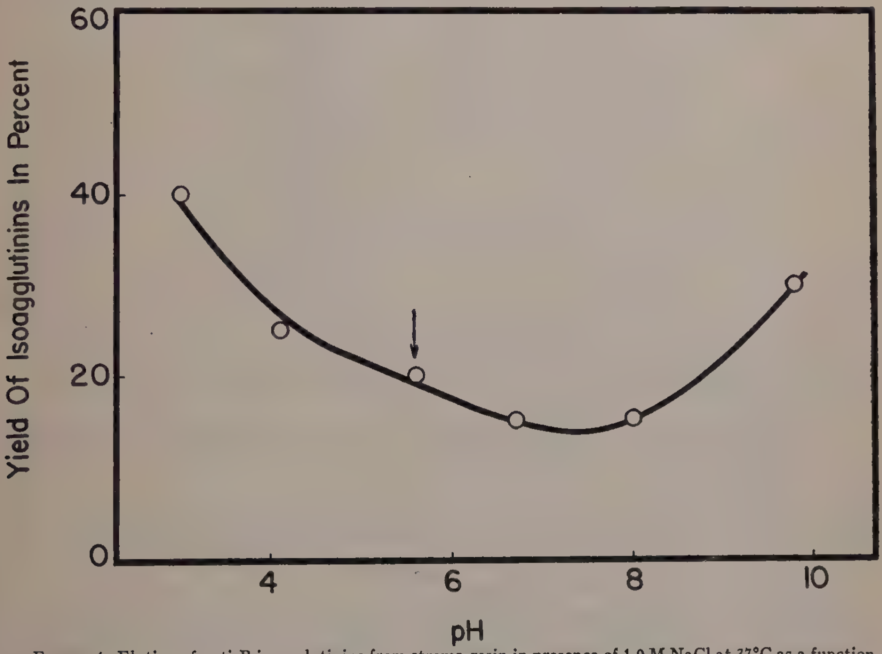

FIGURE 4. Elution of anti-B isoagglutinins from stroma-resin in presence of 1.0 M NaCl at 37°C as a function of pH.

The yield of antibody was improved by an increase in ionic strength (FIGURE 5) and adjusting the pH to 6.1. Under optimal conditions, 55 per cent of the adsorbed antibody was recovered. Five to ten per cent additional antibody could be extracted by a second elution. In purifying anti-B isoagglutinins, different yields were obtained when using A or O plasma as starting material.[18] Stroma-resin derived from A or B red blood cells could be regenerated after elution by treatment with 0.1 N acetic acid. While the adsorption capacity of the regenerated resin was diminished, as compared with unused stroma-resin, the percentage of antibody recovered was slightly increased. It appears from this finding that the first portions of the antibody contacting the stroma-resin are bound tighter than the last portions.

TABLE 3

SPECIFIC AND REVERSIBLE INTERACTIONS OF PLASMA PROTEINS WITH CARBOHYDRATES

Protein	Interacting carbohydrate	Adsorbent	Inhibitor	
			Sugar	Moles/liter*
Isoagglutinins	blood group polysaccharide	red blood cells a) native	N-acetyl-D-glucosamine	0.16
		b) denatured	D-galacturonic acid	0.16
anti-A	A-substance	c) stroma	D-glucuronic acid	0.16
anti-B	B-substance	d) stroma-resin	D-galactose	0.25
(anti-Rh)	(Rh factor)		D-mannose	0.25
			lactose	0.31
			D-glucosamine	0.31
			sucrose	0.75
			maltose	no inhibition
			L-fucose	no inhibition
			D-galactosamine	no inhibition
Amylase†	starch	starch	maltose	0.28

* Lowest concentration of sugar which inhibits agglutination of red blood cells by isoagglutinin; dilutions made in isotonic phosphate buffer.

† Data from Brown, R. K.[23] Glucose and sucrose do not inhibit the interaction of starch and amylase.

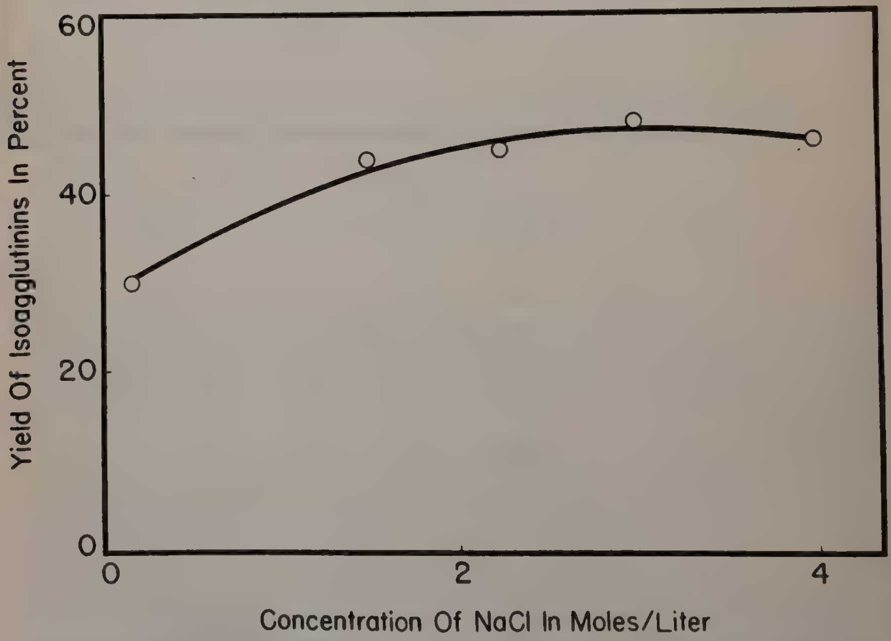

FIGURE 5. Elution of anti-B isoagglutinins from stroma-resin in presence of 0.3 M lactose at 37°C pH 7.2 as a function of ionic strength.

The purity of the dissociated antibody depends naturally on the purity of the starting material, both antibody and antigen. Equally important are the washing of the specific precipitate and the removal of the antibody-releasing agent. In small batches the sugar was separated by dialysis against saline. In a few instances the antibody was precipitated from the eluate with zinc acetate at pH 6.5, the solubility minimum of isoagglutinins. The marked effect of the ionic strength is demonstrated in FIGURE 6. The concentration of anti-B isoagglutinins purified by the described method was 350-fold as compared to plasma. In the purified solution 75–90 per cent of the protein was identified as specific antibody. The highest concentration thus far reported by non-specific fractionation methods was 16-fold.[2]

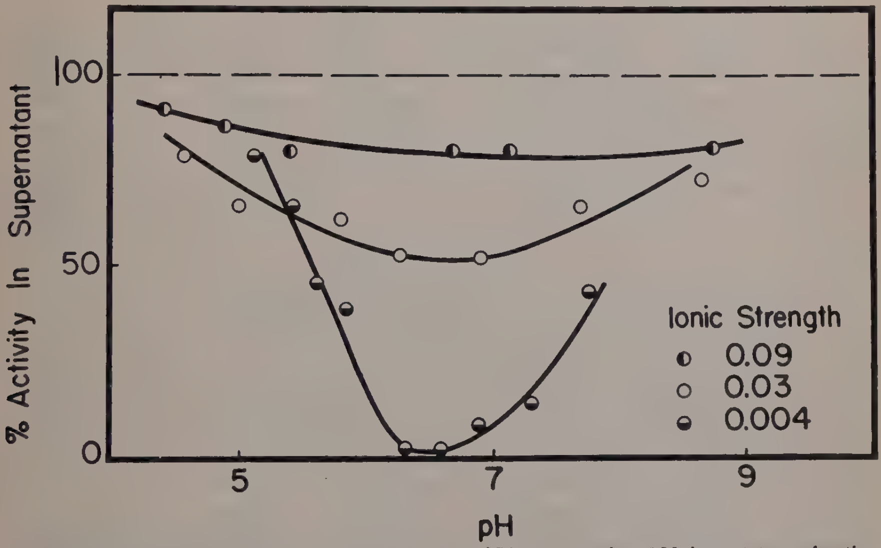

FIGURE 6. Solubility of anti-B isoagglutinins in water at 0°C in presence of 0.005 M zinc acetate as a function of pH and ionic strength.

Preliminary experiments were carried out to purify Rh antibodies. While saline antibodies were precipitated completely by 15 mM zinc glycinate, blocking antibodies were carried down only to a small extent under these conditions. This finding suggests a way for the ultimate separation of the two types of antibodies. The removal of zinc from the antibody was achieved by a cation exchange resin of the carboxylated type, XE 64. The physical chemical properties of the purified antibodies will be described elsewhere.

B. Elution of antibodies to protein antigens. In systems where the antigen is a protein, no antibody has been recovered from specific precipitates by using the sugars employed for the elution of isoagglutinins. Sodium chloride up to concentrations of 4 M and glycine up to 3 M at 37°C are equally ineffective for the dissociation of such specific precipitates.

We have resorted to a method using acid as an eluting agent.[5,7] From the complex of human serum albumin-resin with horse anti-serum (TABLE 1, resin 3) 25 mg. protein were dissociated at pH 3.6, 21 mg. of which were specifically precipitated by human serum albumin.

In the case of influenza virus A, antibody has been recovered from the virus-resin by applying the same procedure. The eluates inhibited the agglutination of chicken red blood cells by the virus completely up to a dilution of 1:16, partially up to 1:128. In this particular case (TABLE 2, resin 3) the recovery of antibody amounted to 10 to 20 per cent while the concentration of antibody on a nitrogen basis was of the order of 150. Considering the inaccuracy of the hemagglutination test, this is an approximation and the experiments have been controlled by using the mouse protection test.

Discussion and Summary

One of the trends in developing ion exchange resins has been to adapt a resin to a particular use by the introduction of various groups and crosslinkages. A great number of new resins, which have been made available, have a very specific configuration and their use is limited to one special type of operation. Such resins have been referred to as tailor-made and have been found useful in biological systems.

An extreme specificity has been conferred on resins by linking antigens irreversibly to them. By this procedure, the resin has been wholly or partially deprived of its ion exchange properties. Instead of exchanging ions, the antigen-resin adsorbs but one substance, specific antibody, which can be eluted to a varying extent. In the case of the ABO blood group system, the antigen-resin can be re-used after elution of the antibody.

Advantages in preparing an insoluble antigen are apparent in all four phases of the procedure listed in the introduction: (1) the removal of antibody from the starting material can be carried out continuously, its separation is not bound to the time-consuming flocculation and centrifugation of a specific precipitate; (2) the washing phase is more effective due to reduced occlusion of non-specific protein on the antigen-film coating the resin beads; (3) the dissociation is facilitated as the forces between antigen and antibody are weakened by the linkage of the antigen to the resin; (4) the separation of released antibody from the antigen is effected together with phase 3.

The mechanism of the elution of isoagglutinins with sugars is open to question. The fact that those sugars which naturally occur in blood group substance are effective in eluting antibody suggests a specific action, in the sense that the sugar combines with the agglutinin to form a hapten-antibody complex. An inhibition by a simple sugar, such as glucuronic acid or glucuronides, has been observed in systems of pneumococcus polysaccharides which contain large amounts of glucuronic acid.[19] In those cases the concentration of the uronic acid necessary to bring about inhibition of the antigen-antibody reaction was considerably smaller than the concentration of the sugars used in our experiments. The finding that fucose, which appears to be an end-group of the blood group polysaccharide,[20] has almost no inhibitory effect, (TABLE 3) is also against a hapten hypothesis. Furthermore, amino acids, such as histidine, lysine, and combinations thereof, which were suggested as possible determinant groups of the polysaccharide antigen,[21] do not inhibit the reaction of red blood cells with the corresponding isoagglutinin. There is no evidence that the inhibiting power of the sugars listed in TABLE 3 is related to their molecular size, as was found for the inhibition of amino acids and other compounds in the system egg albumin–anti-egg albumin.[22]

Previously, an enzyme had been purified by Brown[23] in this laboratory by making use of a procedure very similar to the described purification of isoagglutinins. Serum amylase was adsorbed on its substrate starch and was eluted by maltose, an end product of hydrolysis of starch.

No method has been found to elute antibodies from protein antigens at neutral pH. Preliminary experiments have suggested the use of polyelectrolytes. There is much evidence that the tightness of antigen-antibody complexes is due to a great number of relatively weak bonds rather than to a few strong linkages.[24] Maleic anhydride-styrene copolymer exhibits a stronger affinity for serum albumin than for γ-globulin;[25] added to a specific precipitate of serum albumin-anti-serum albumin, the polyelectrolyte may displace the antibody by competing with the latter for the combining sites on the antigen. The interactions of other polyelectrolytes and resins of very low degree of crosslinkage (1–2 per cent) with viruses and antibodies are under investigation.

Acknowledgment

I wish to express my gratitude for the help and encouragement given me in this work by Doctors E. J. Cohn, J. T. Edsall, and J. L. Oncley.

Furthermore, I am indebted for helpful advice on special aspects of this work to Doctors L. K. Diamond, H. M. Dintzis, J. Enders, W. L. Hughes, R. Kunin and D. M. Surgenor, and to Miss J. Sullivan for performing the hemagglutination tests.

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CONSIDERATIONS IN THE SELECTION OF CATION EXCHANGE RESINS FOR THERAPEUTIC USE

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The concept of using cation exchange resins to remove sodium from the body was first established by Dock in 1946.[1] Although this initial study was made in rats, the basic principle was shown to be correct. The low exchange capacity of resins then available, however, made it unfeasible to attempt to carry out therapeutic trials in human subjects simply because a 200 gm. daily dose of that resin would be required to give equivalent effects in man. Practical utilization of Dock's findings depended, therefore, on finding a cation exchange resin of greater capacity. As to a reasonable dose, various clinicians hazarded a guess that 75 g. would be the maximum amount which a patient would be willing to ingest day after day.

In the face of this limitation the ultimate therapeutic application of the cation exchange idea depended on (1) the disclosure of new resins possessing a greater cation binding capacity and (2) test methods for the quick recognition of the more promising new resins. Several groups set up some sort of *in vitro* test method and then went on to *in vivo* tests in the hope of establishing a correlation between both methods.

Only a few resins were available but from among them have come four commercial products which serve as adjuncts in the treatment of edematous states due to sodium retention. Among the clinicians who have given resin therapy a reasonable trial, there is quite general agreement as to its effectiveness and that it fills a need in modern medical practice.

None of the known resins fully meets the specifications of the ideal preparation. This accounts, to a large extent, for the fact that some patients are unwilling, or unable, to tolerate the poor palatability and/or gastrointestinal distress long enough to become adapted to this type of medication. These problems are attributable in large measure to the high dosage and to the texture which is variously described as gritty, or chalky, or sandy. The answer to these difficulties lies in finding polymers better suited to this application. I should like, therefore, to discuss some of the problems, methods and results incidental to this objective.

Before proceeding further, however, I should like to extend our thanks to Rohm and Haas, Chemical Process Company, B. F. Goodrich Chemical Company, National Aluminate Company, Permutit Company, Monsanto Chemical Company, the Algin Corporation, and Professor G. B. Butler of the University of Florida, who supplied the polymers used in these studies.

The ideal resin, in our opinion, should have high binding capacity, wide utilization of inherent capacity at pH 6–8, and a favorable sodium-potassium capacity ratio. In addition, it must also be non-toxic, palatable, and inexpensive.

Now, what are the shortcomings of the resins used in the present therapeutic products? First of all, the dosage is too high. This is due, in the case of the sulfonic resin, to too low a capacity, while the chief disadvantage of the carboxylic resin is that so little of its inherent capacity is actually used. FIGURE 1, for instance, illustrates typical titration curves for three different classes of resin. You will note that the capacity of the sulfonic resin is already maximal at pH 4, whereas, the carboxylic resin, with a much higher total capacity, is only partly utilized at pH's which are likely to be found in the gastrointestinal tract. If the carboxylic curve could be depressed about 2–3 pH's, the dose of resin could probably be cut in half. The phosphonic resin is intermediate in acid strength.

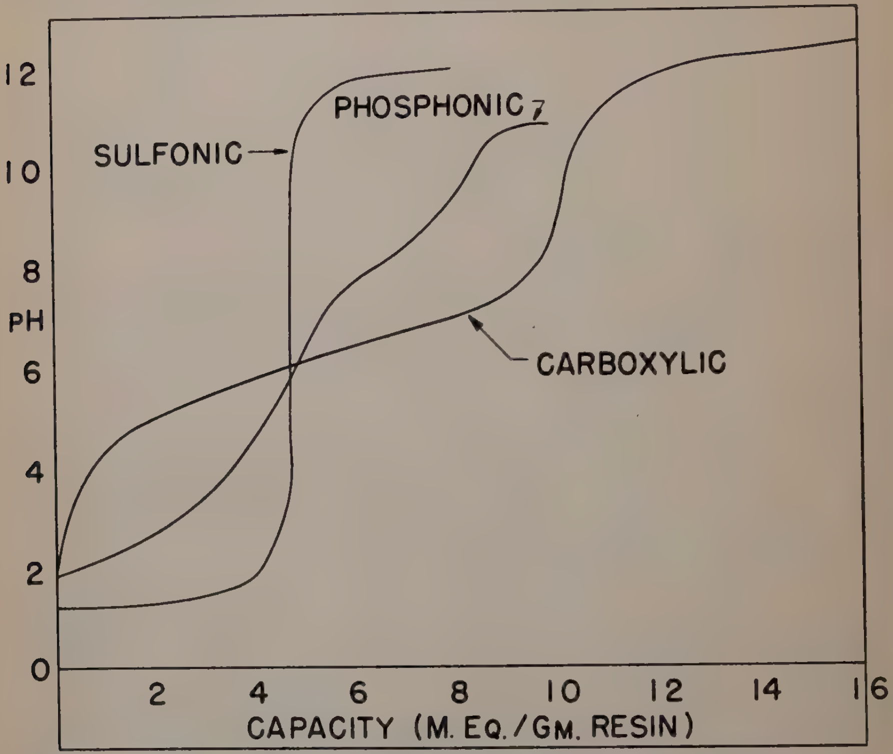

FIGURE 1. Typical titration curves for sulfonic, carboxylic, and phosphonic type cation exchange resins.

Another drawback with present resins is that they bind too much potassium, thereby blocking exchange groups which would otherwise be available for sodium removal. Finally, resins today are too expensive to permit their most widespread use.

With the knowledge of both the desirable properties and the shortcomings of present resins, the polymer chemist then attempts to tailor-make new resins more suitable for therapeutic purposes. As a result of these efforts, we have been faced with the problem of examining over one hundred new polymers. To evaluate these intelligently, some sort of screening procedure is obviously necessary.

After a rather wide experience in developing a screening technique, this is the way we approach the problem. Our first step employs *in vitro* capacity tests by which we determine not only total (maximum) capacity but also the capacity at pH's 6, 7, and 8. Since the actual intestinal pH cannot be expressed as a single, absolute value, we prefer to use these three pHs.

To determine total capacity, resins are first ground to a fine powder and one gram is shaken for four minutes with 200 ml. of N/10 NaOH. The supernatant solution is then back-titrated and the bound sodium is calculated.

The capacity at pHs 6, 7, and 8 is found by shaking 1.0 g. of resin for four minutes in 50 ml. of McIlvaine's buffers, adjusted with NaCl to contain 26 mEq. of sodium. The resin is filtered off and washed, following which the sodium is eluted with HCl and determined by flame photometry.

Sodium-potassium ratios are found by shaking 1 gram of resin with 50 ml. of solution containing 13 mEq. each of potassium and sodium chlorides, buffered at pH 7. Bound sodium and potassium are determined by elution and flame photometry.

Perhaps you may question whether the resins actually reach equilibrium in four minutes. It is true that they do not in the sense that the physical chemist thinks of it. We have found, however, that if the resin is pulverized, even the slowest one comes so near to equilibrium in this period of time that the error is well within our ability to predict what will happen when the resin is used *in vivo*.

You will note that our medium contains no calcium, magnesium, or any cations other than sodium and potassium. Nor do we feel that use of the so-called artificial gastric or intestinal juices would tell us very much at this point since the actual composition of the contents of the G.I. tract is, in any given individual at any given time, wholly unknown. We prefer to get a quick empirical capacity picture and then go on to *in vivo* testing as rapidly as possible.

When various laboratories started "tailor-making" polymers for our consideration, we found that the above methods were not always applicable. For instance, soluble polymers and those insoluble polymers which contain an appreciable soluble fraction must be handled differently. We have found that practically all of these can be titrated automatically provided the alkali is added slowly. Capacities are read from the titration curve at pHs 6, 7, and 8, and at the final plateau. Sodium-potassium ratios are not determined for these materials.

TABLE 1 shows some comparative results obtained with some of the classical cat on exchange resins. The sulfonic resins have already attained maximum capacity at pH 6 while the Amberlite XE-96 rises through this range and goes to 10 mEq./gm. at high pHs. In TABLE 2 are given representative data on a few of the newer polymers which have more recently been made available to us.

We would eliminate the first polymer from further testing because of its low capacity. The second polymer does not come up to Amberlite XE-96 for total capacity but its intermediate capacities look interesting. The value at pH 6 is high, at pH 7.0 it is as good as the Amberlite, and at pH 8 it is better.

We would, therefore, take this polymer into our *in vivo* tests. Polymers 3 and 4 obviously would be considered further.

The sodium-potassium ratios of several polymers are shown in TABLE 3. Polymer 1 is of no further interest from the point of view of total capacity or sodium-potassium ratio and hence would be eliminated. Polymer 2, in spite of its lower total capacity, would merit further testing because it shows a preference for sodium over potassium. Since Polymer 3 shows up well in both respects it would be reserved for additional study. Thus, of a series of the first 61 polymers examined, 47 were rejected on the basis of this primary screening and 14 were put into the second screening. The question may be raised as to whether we might overlook a good resin by this procedure. This is a risk with all biological screening procedures. From the practical standpoint, however, the risk is small and is justified by the speed and economy of effort.

Table 1

Results of Testing Various Resins by Primary in Vitro Capacity Test

Resin	Capacity mEq./Gm.		
	pH 6	pH 7	pH 8
Sulfonic 6	4.3	4.2	4.4
Sulfonic 7	4.5	4.7	4.4
Sulfonic 8	5.0	5.0	4.8
Sulfonic 9	5.2	5.3	5.4
Carboxylic (Amberlite XE-96)	1.7	5.0	5.9

Table 2

In Vitro Capacity Results Obtained with Several Newer Exchange Polymers

Polymer	Capacity mEq./Gm.			
	Total	pH 6	pH 7	pH 8
1	4.1	2.3	2.9	3.7
2	9.5	4.5	5.0	6.4
3	11.9	3.3	6.4	9.4
4	13.6	7.3	11.7	12.8
Amberlite XE-96	10.0	2.6	5.0	5.5

Table 3

Na:K Capacity Ratios of Various Typical Ion Exchange Polymers

Polymer	Total Capacity	Na:K (pH 7)
1	5.6	0.8
2	8.1	1.6
3	11.8	1.2
Amberlite XE-96	10.0	1.0

Our next step is to test the polymers in animals. This is achieved by feeding a group of 10 rats a mixture of 90 per cent pulverized Purina Dog Chow plus 10 per cent polymer. In the case of soluble polymers, we use the dry, powdered polymer in the hydrogen form. This diet is fed to rats for 5 days during which time the food intake is recorded. Stools of the 10 rats are collected and pooled during the five day period. They are then ashed, and extracted with N HCl. Sodium and potassium are determined and expressed as mEq. of cation per gram of resin ingested.

Some typical results are shown in TABLE 4. As with the *in vitro* testing, our basis for comparison is Amberlite XE-96.

As a result of this test, Polymer No. 1 would likely be tested further if its physical properties were such as to suggest that it might prove to be better tolerated by the patient. Polymer No. 2 would go on to the next test because of its increased sodium removal capacity. Polymer No. 3 in this table, evincing no further interest, would be eliminated at this point.

In the next step capacity in dogs is determined. Originally this was our only biological test. In the early days of the problem, it was possible to apply this test to all resins passing the *in vitro* test because there were so few materials available. We regard this test as our most reliable one. It was by means of this procedure that we originally predicted that the dose of the presently used resins would be from 45–50 grams per day. The dose eventually settled on by the four companies now marketing a resin product was 40 grams by one, 45 grams by two, and just under 45 grams by the other.

TABLE 4

COMPARISON OF IN VIVO CAPACITY BY RAT SCREENING TEST

Polymer	Food Intake	Na Excretion mEq./Gm.	K Excretion mEq./Gm.
1	11.0	1.0	0.7
2	13.6	1.2	0.9
3	19.3	0.7	—
Amberlite XE-96	19.4	1.0	0.4

In our dog test, we remove the food from the cage at five p.m. each day. The next morning the dog is offered a meal of 350 g. canned dog food, 50 ml. of milk and either 12.5 g. or 25.0 g. of powdered resin, all intimately mixed. A ten kilo dog will eat this meal completely. At the beginnning of the test, and every three days thereafter, the dog receives a capsule of carmine to serve as a marker in the stools. Stools are collected and separated into three-day periods. They are then analyzed for sodium and results are expressed as mEq. of sodium excreted per day.

Typical values obtained by this method using Amberlite XE-96 are presented in TABLE 5.

We use the 25 g. dose wherever possible but sometimes due to a limited supply of polymer we must cut the dose to 12.5 g. The results at this lower dose average out pretty well to about half the value obtained with the 25 g. dose. A large number of such tests show an average value close to 30 mEq./day at the 25 g. dose level. The third line illustrates an interesting point which is sometimes observed. In this case, during the first period only 18 mEq. of sodium/day was removed, but, during the second period, this rose to the expected range. In such a case, if one continues the experiment, he invariably finds that, during the third and fourth periods, the values run close to 30 mEq./day. We disregard such an abnormally low value in the first period, in view of this experience. Moreover, we usually run a minimum of two dogs on a resin, and we have never had this happen in both dogs in the same test.

During the course of these *in vivo* tests we can learn a good deal about the potential acceptability of the resins. For instance, the food consumption in the rat test often serves as a cue that a particular resin may be especially unpalatable. Sometimes the dogs vomit, develop a severe diarrhea, or refuse to eat, which alerts us to possible difficulties with a new material.

We have found that, where polymers are intended for *in vivo* testing, it is advisable for the polymer chemist to use the best grades of starting materials and to pay special attention to the purification of the resulting polymer. In some cases of intolerance to a resin, the trouble can be traced to unreacted starting materials or impurities in the starting materials.

TABLE 5
CAPACITY OF AMBERLITE XE-96 BY IN VIVO DOG TEST

Resin intake (Gms.)	Fecal Sodium (mEq./Day)		
	Control	First Period	Second Period
12.5	3.8	12.6	18.4
25.0	3.6	32.4	27.3
25.0	4.1	18.1	31.6

TABLE 6
THE TOXICITY IN RATS OF VARIOUS MONOMERIC MATERIALS COMMONLY USED FOR THE SYNTHESIS OF POLYMERS

Monomer	Approximate LD_{50} (mg./kg.)
Acrylic Acid	3000
Methacrylic Acid	3000
Divinyl Benzene	7500
Maleic Anhydride A	900
Maleic Anhydride B	2000

Because of these polymers, we have run toxicity and tolerance tests on some of the starting materials using a grade commonly employed for polymerization work. TABLE 6 gives the LD_{50} in rats of some of these compounds. Actually, from the toxicity viewpoint, most of these compounds are not too objectionable. You will note, however, the difference in the two samples of maleic anhydride. TABLE 7 shows how dogs tolerate these same materials. The endpoint used in this test was vomiting, diarrhea and/or refusal to eat. It is interesting to note that, although the LD_{50} of acrylic and methacrylic acids in rats was the same, dogs could tolerate only one-quarter as much acrylic acid as they did methacrylic acid.

Before proceeding to human studies of short duration for the purpose of evaluating the sodium removing capacity, rats are fed the material at a 10 per cent level of the diet for 30 days. They are observed daily for signs of toxicity and tolerance. At the end of thirty days, all animals are sacrificed and the major organs and tissues are subjected to a gross and microscopic examination for evidence of pathology, *i.e.* symptoms, due to the drug.

In addition to our active interest in the problem of general screening, we have also been engaged in trying to establish some principles and leads which might give direction and guidance to the problem of "tailor-making" polymers. In this connection we were surprised at the toxicity of some of the soluble polymers. Early data suggested that there might be an optimum range of molecular sizes, as judged by intrinsic viscosity values, above and below which polymers would be toxic. The initial observations which suggested this possible relationship are indicated in TABLE 8 by the asterisks. We, therefore, extended the series to include polymers of higher and lower intrinsic viscosity. It is apparent from the data that this generalization was not justified.

TABLE 7

THE TOLERANCE OF DOGS TO VARIOUS MONOMERIC MATERIAL COMMONLY USED IN THE SYNTHESIS OF EXCHANGE POLYMERS

Monomer	Tolerated Dose (Gms.)
Acrylic Acid	0.5
Divinyl Benzene	1.0
Maleic Anhydride A	1.0
Maleic Anhydride B	1.5
Methacrylic Acid	2.0

TABLE 8

TOXICITY OF SOLUBLE POLYMERS OF VARYING INTRINSIC VISCOSITY

Polymer	Intrinsic Viscosity	No. Deaths/No. Tested
1	0.52	6/18
2*	0.92	4/4
3*	1.32	0/8
4*	1.56	5/8
5	1.64	1/8
6	1.98	2/8
7	2.36	4/8

Another avenue of exploration stemmed from the supposition that, since the sulfonic resins probably act predominantly in the stomach while the carboxylic resin acts in the intestine, perhaps a combination of the two types of resins would be more effective than the individual components. The results of such a trial are shown in TABLE 9. In this dog experiment three different mixtures of sulfonic and carboxylic resins had the same sodium removing capacity as would be expected of the individual resins alone. We have also tested two resins which contained both sulfonic and carboxylic groups in the same molecule but there was no evidence of increased activity.

We have also been interested in the effect that varying degrees of crosslinking might have on *in vivo* sodium removal. *In vivo* testing of polymers crosslinked approximately 0, 0.1, 1.0 and 10 per cent (TABLE 10) showed that there was little or no effect on sodium removal. However, at the lower degrees of crosslinking the potassium removal was markedly increased. Thus, although the total *in vivo* capacity was enhanced it accomplished nothing in terms of better sodium removal.

Some months ago, Ludwig, *et al.*,[2] reported that a naturally occurring carboxylic acid polymer, namely alginic acid, had a suffiiciently high *in vitro* capacity to justify biological testing. Our attention was aroused, because alginic acid titrates in some respects like a strong acid (FIGURE 2). Its capacity rises rapidly with increasing pH and then forms a plateau. It rises again at higher pHs. The plateau is in the same range of activity as that of the sulfonic and carboxylic resins at *in vivo* pHs. We tested this substance in dogs and found that the capacity starts off at a very high level during the first period but drops markedly in subsequent periods (TABLE 11). These same authors reported similar *in vitro* results with CMC but the sodium binding capacity in dogs was much inferior to that of currently used resins. Subsequent work showed that, in humans,[3] alginic acid had only ⅕ to ⅙ the activity as the carboxylic type resins. It has been suggested that this poor result in humans resulted from hydrolysis of the polymer in the gastrointestinal tract.

TABLE 9

THE IN VIVO CAPACITY OF COMBINATIONS OF SULFONIC-CARBOXYLIC CATION EXCHANGE RESINS

Composition		Dose (Gms.)	Fecal Sodium (mEq./Gm.)	
COOH %	SO_3H %		First Period	Second Period
75	25	25	28.9	34.0
75	25	25	31.0	31.2
50	50	25	35.3	34.0
50	50	25	32.0	19.1
25	75	25	28.8	31.4
25	75	25	26.3	34.5

TABLE 10

THE EFFECT OF CROSSLINKING ON CAPACITY OF CATION EXCHANGE POLYMERS IN RATS

% Crosslinking	NA Excretion (mEq./Gm.)	K Excretion (mEq./Gm.)
0	1.0	0.7
0.1	1.1	1.3
1.0	1.1	1.4
10.0	1.0	0.4

Finally, there are the phosphonic resins to consider. They are of interest because they appear to be intermediate in acid strength between the sulfonic and carboxylic resins. Moreover, *in vitro* testing indicates a favorable sodium-potassium ratio (TABLE 12). These two resins when tested in rats, however (TABLE 13), showed a very much reduced capacity to remove sodium from the animals when compared to Amberlite XE-96. Potassium excretion remained unchanged.

From our experience we may conclude that the field of opportunity to improve the presently available polymers is still wide open. Although effective,

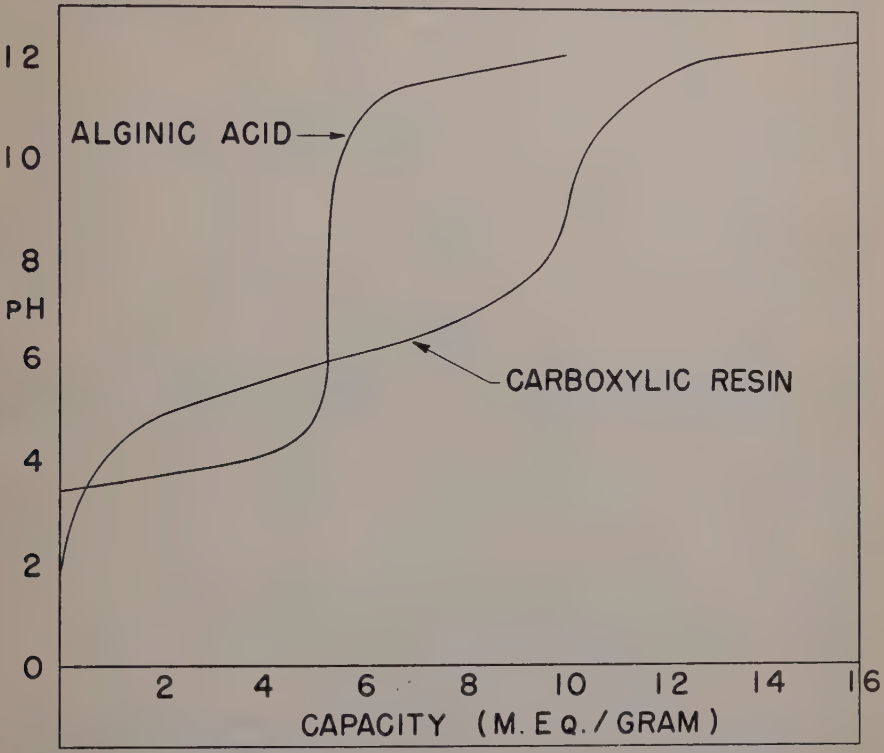

FIGURE 2. Typical titration curves for alginic acid and carboxylic type cation exchange resin.

TABLE 11

THE IN VIVO CAPACITY OF ALGINIC ACID AND CMC OBTAINED BY THE DOG TEST

Dose (Gms.)	Period			
	First	Second	Third	Fourth
		Alginic Acid		
25	44.2	24.7	—	—
25	47.3	35.0	—	—
25	37.0	30.0	30.7	32.3
25	34.5	19.9	24.9	19.3
		CMC		
25	22.6	21.1	—	—

within their limitations, for the removal of sodium from edematous patients, these polymers fall short of meeting the ideal requirements imposed by human therapeutic applications. Therefore, continued efforts should be directed toward the development of the ideal polymer. Only by examining new polymers of varying nature, can we hope to learn enough about the fundamental relationships in this field to guide the polymer chemist in his efforts to fill our precription.

TABLE 12
THE IN VITRO CAPACITY OF TWO PHOSPHONIC RESINS

Resin	Capacity (mEq./Gm.)			
	Total	pH 7	pH 8	Na/K
A	5.0	4.7	4.7	1.4
B	8.1	4.2	4.2	1.2

TABLE 13
THE IN VIVO CAPACITY OF PHOSPHONIC RESINS IN THE RAT TEST

Resin	Food Intake	Na Excretion mEq./Gm.	K Excretion mEq./Gm.
A	18.3 gms.	0.39	0.38
B	20.2 gms.	0.52	0.38

References

1. DOCK, W. 1946. Trans. Assoc. Am. Physicians. **59**: 282.
2. LUDWIG, B. J., *et al.* 1952. Proc. Soc. Exptl. Biol. Med. **79**(1): 176–179.
3. FELDMAN, H. S., *et al.* 1952. Proc. Soc. Exptl. Biol. Med. **79**(3): 439–41.

Discussion of the Paper

F. C. NACHOD, *Sterling-Winthrop Research Institute, Rensselaer, N. Y.*: In the evaluation of exchange materials to be used in a problem like the present one it is of importance that one understand, at least in part, the thermodynamic and kinetic factors that are significantly different for the two types of resin which have been studied in the sodium diversion field.

The ultimate capacity of a resin is often used as an argument for its application, however, without proper reference to the pK of its functional group. FIGURE 1 in Heming and Flanagan's paper shows the titration curves for the sulfonic and the carboxylic resins. While it can be seen that the carboxylic resin may exhibit a capacity of 12 mEq./g. it must be noted that this is only possible at pH of 11.8, *i.e.* not under physiological conditions. At a low pH, say 5, the capacity is only 2 mEq./g. at best.

A convenient method of demonstrating the capacity of a given exchange material is the Freundlich isotherm

$$\frac{x}{m} = Kc^{1/p}$$

where $\frac{x}{m}$ is the amount exchanged per weight of resin, c the concentration and K and p constants, characteristic of the system (K) and the resin (p). Experiments carried out in our laboratories demonstrate that the sulfonic resin both

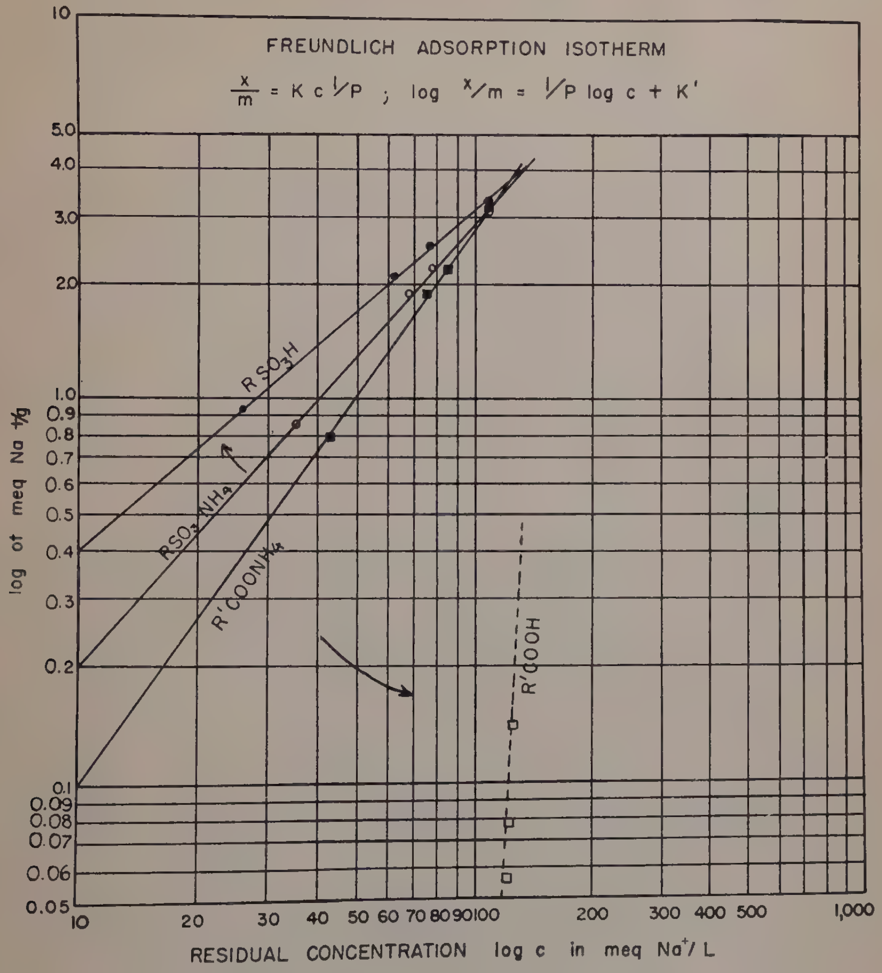

FIGURE 1.

in ammonium and hydrogen forms gives isotherms of lower slopes, or a more favorable exchange picture in the sodium exchange than the carboxylic resin (FIGURE 1).

Finally the kinetics of the reaction are an important facet in our consideration. FIGURE 2, taken from data of Dr. Kunin, indicates that, in the hydrogen alkali exchange, a 90 per cent complete reaction is attained in one minute for the sulfonic resin while the carboxylic resin requires a 200-fold longer time.

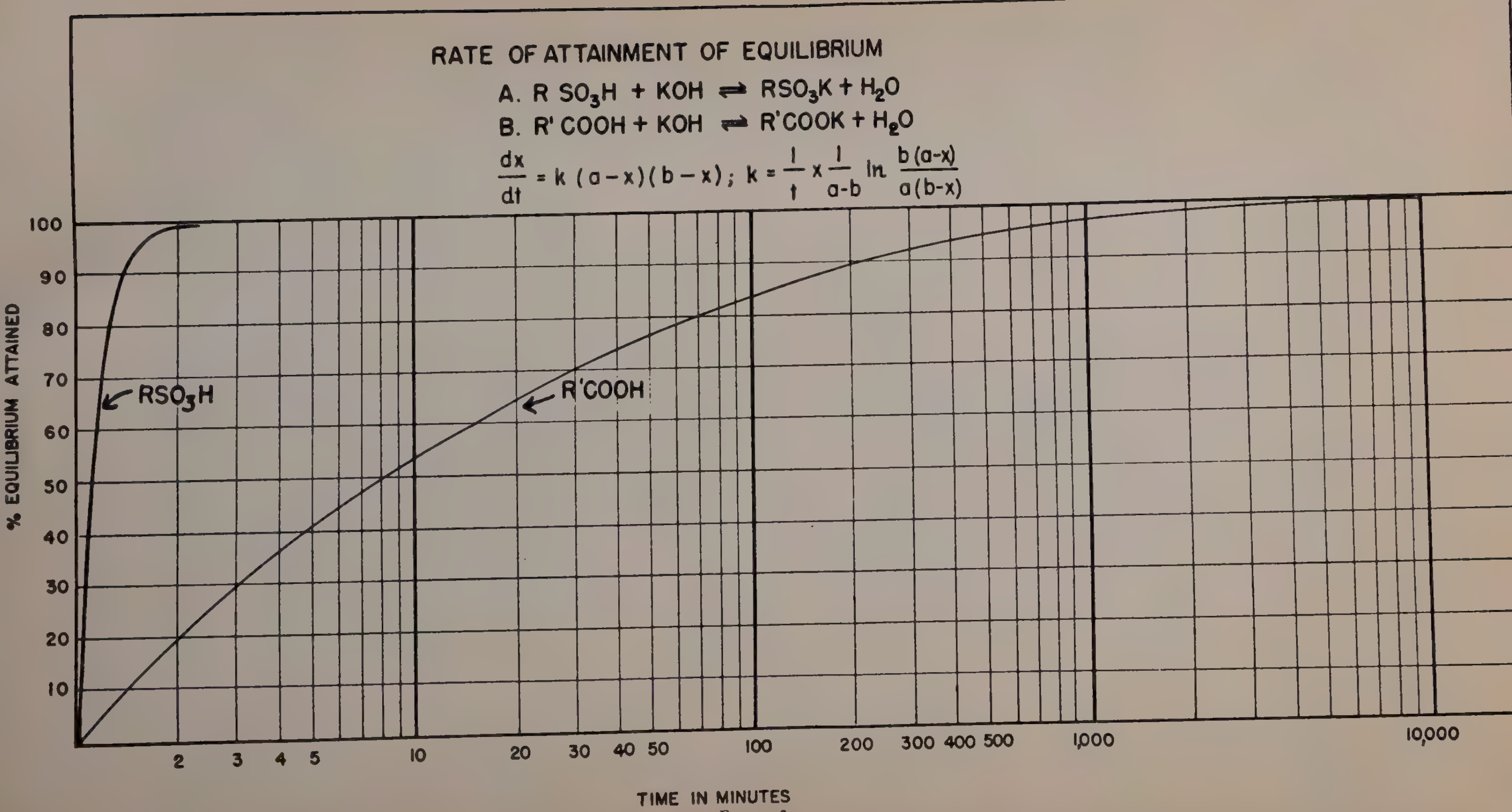
RATE OF ATTAINMENT OF EQUILIBRIUM
A. R SO_3H + KOH ⇌ $RSO_3K + H_2O$
B. R' COOH + KOH ⇌ R'COOK + H_2O
$\frac{dx}{dt} = k\,(a-x)(b-x)$; $k = \frac{1}{t} \times \frac{1}{a-b} \ln \frac{b(a-x)}{a(b-x)}$
RSO_3H
R'COOH
100
90
80
70
60
50
40
30
20
10
% EQUILIBRIUM ATTAINED
2
3
4
5
10
20
30
40
50
100
200
300
400
500
1,000
10,000
TIME IN MINUTES

FIGURE 2.

If we consider that in the stomach the resins are present in the hydrogen form it can immediately be seen that the carboxylic resin has no chance for any exchange of ions at that site.

These comments of course are only theoretical considerations, but are amply borne out by Dr. McChesney's review and more recently by Doctors Whiting and Millar's *in vivo* results (W. B. Whiting and M. S. Millar. 1952. Texas State J. of Med. **42:** 585.).

SOME ASPECTS OF CATION EXCHANGE RESINS AS THERAPEUTIC AGENTS FOR SODIUM REMOVAL

By E. W. McChesney, F. C. Nachod and M. L. Tainter
The Sterling-Winthrop Research Institute, Rensselaer, N. Y.

Since Dock's publication in 1946,[1] about fifty papers dealing with the use of cation exchange resins for restricting sodium absorption have appeared.[2,3] Approximately 75 per cent of these papers have dealt with studies in human subjects and, as a result, the potentialities, limitations, and hazards[4,5] of the procedure are quite well defined. It is clear that a valuable therapeutic medicament, although one which requires thorough understanding if it is to be effectively and safely used, has become available.

Several types[6] of exchange resins can be employed to produce changes in cation metabolism. These resins differ with respect to both matrix and functional groups. Two types of functional groups, the sulfonic and the carboxylic, have been extensively studied in this connection. It is the object of this paper to discuss critically the inherent differences in behavior of these two materials, specifically: (1) their relative efficiency in the removal of sodium from the alimentary canal; (2) the effect of the sodium and potassium levels in the diet on their cation uptake; and (3) other considerations related to their therapeutic usefulness.

If one sets out to evaluate a new therapeutic agent, the chronic toxicity problem must be taken into account. We have carried out in our laboratories an extensive long-term feeding experiment in addition to many short-term studies. FIGURE 1 shows growth curves of some of the animals on the long-term experiment, up to and including its 65th week. This is a length of time which represents approximately one-third the life span of the rats. The results indicated no significant decrease in the rate of growth at resin levels of 10 per cent of the diet. Details of this work will be published at a later date.

A comparison of *in vivo* uptake of sodium, potassium, and total alkali metal ions by sulfonic and carboxylic resins is shown in TABLE 1. It indicates a 25 per cent better average uptake by three sulfonic preparations than with the three carboxylic formulations. It also shows about equal potassium removal, and a better selectivity coefficient of the sulfonic type for sodium.

Much has been said about the higher *in vitro* capacity of the carboxylic resins (about 12 meq./g.) in contrast to the sulfonic types (about 5 meq./g.). It should be remembered, however, that the full capacity of the carboxylic types is realized only at pH values above 10, due to the weak ionization constant of the functional group. Under physiological conditions this high "theoretical" capacity is not available. Hence, an efficiency ratio, which we could define as the *in vivo* capacity for sodium plus potassium divided by the maximum theoretical capacity, yields values of 0.33 and 0.11 for the two types, respectively, *i.e.* a greater efficiency or saturation of the sulfonic resin. The same conclusion with respect to superiority of sulfonic over carboxylic resins as therapeutic agents was reached by Herken and Wolf,[3] who stressed the advantages of the relative insensitivity to pH and the greater reaction rate of the sulfonic type.

In addition to the foregoing considerations, the following factors must be taken into account in evaluating therapeutic usefulness: (1) *in vivo* capacity in clinical trials; (2) removal of other nutrient elements, vitamins, etc.; and (3) patient acceptance as influenced by taste, incidence of nausea, sense of fullness, tendency to produce constipation, *etc.*

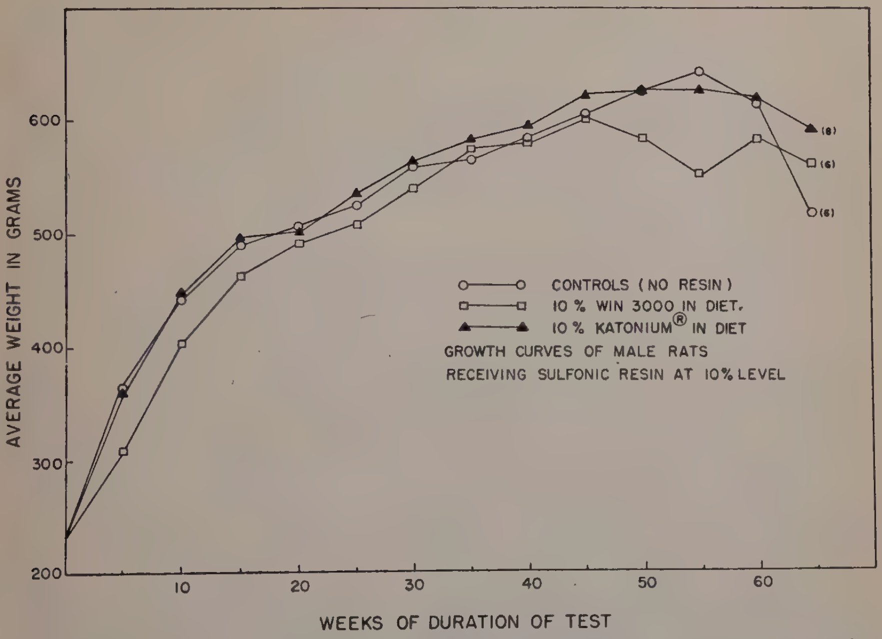

FIGURE 1. Growth curves of male rats during test of chronic toxicity of sulfonic resin. Numerals in parentheses represent the number of survivors at the 65th week (there were ten animals in each group, initially). Win 3000 is ammonium sulfonate resin, and Katonium® is a 75/25 mixture of ammonium/potassium sulfonate resin.

TABLE 1

COMPARATIVE EFFICIENCY OF CATION EXCHANGE RESINS FED AT 10% LEVEL

Sulfonic Resins				Carboxylic Resins			
Product	*In vivo* uptake, meq/g.av.±s.d.			Product	*In vivo* uptake, meq/g. av.±s.d.		
	Na	K	(Na±K)		Na	K	(Na±K)
Resin A........	1.28 ± 0.11	0.39 ± 0.06	1.67 ± 0.07	Resin D.......	0.90 ± 0.13	0.28 ± 0.07	1.18 ± 0.20
Resin B........	1.04 ± 0.14	0.36 ± 0.10	1.40 ± 0.16	Resin E*......	0.86 ± 0.16	0.42 ± 0.09	1.31 ± 0.22
Resin C........	1.05 ± 0.14	0.43 ± 0.13	1.48 ± 0.22	Resin F.......	0.92 ± 0.17	0.41 ± 0.08	1.33 ± 0.07
Average........	1.12	0.39	1.52	Average.......	0.89	0.37	1.27

Basal Diet provided 28 meq. Na and 17 meq. K per 90 g. consumed.
* Values corrected for moisture content.

The number of direct comparisons of sulfonic and carboxylic resins reported in human subjects is not sufficient to warrant definite conclusions as to their relative efficiencies, since most workers have not carried out the metabolic experiments necessary to establish this point. Kahn and Emerson,[7] however, reported the two types to be about equally efficient so far as sodium removal was concerned. Emerson, Kahn, Vester and Nelson[8] also found the ammonium forms of the two resins to be equally efficient in sodium removal, with the carboxylic resin binding more potassium, but the data were obtained with only one subject on each resin. Other workers have frequently expressed preference for one type of resin or the other but have usually based their choices on considerations not related to analytically demonstrated differences in efficiency of sodium removal.

Most *in vivo* studies cover only one of the types. Irwin *et al.*[9] reported on the sulfonic resin (Dowex 50 H) in one essentially normal human subject, and estimated that it took up about 1 meq. each of sodium and potassium per g. The calcium uptake was essentially zero, and magnesium metabolism was not studied. This is a somewhat greater uptake of alkali cations than we have usually found in rats, but it must be kept in mind that their subject's mineral intake was very liberal, and that he was seriously constipated near the end of the resin feeding period. Both of these factors would tend to increase apparent efficiency. Greenman and co-workers,[10] using a carboxylic resin preparation, estimated its sodium uptake as 0.4–1.0, and its sodium-plus-potassium uptake as 1.5–1.9, meq. per g. Although the sodium intakes were generally liberal, some of their cases had virtually no increase of fecal sodium as a result of resin feeding. They also found that a fully saturated sodium resin had a diminished potassium uptake, and *vice versa.* We are in general agreement with these observations, but feel the reservation should be made that the incorporation of as much as 1–2 meq. of potassium *per* g. into either carboxylic or sulfonic resin diminishes its *in vivo* sodium uptake very little.[11] Such incorporation reduces the dangers of potassium depletion.[12]

From several clinical reports dealing only with the carboxylic resin,[8, 13] in which estimates of its *in vivo* efficiency were attempted, it may be concluded that, on a diet providing 500–800 mg. sodium per day (the resin dosage usually being about 45 g. per day), the sodium uptake of the resin is 0.4 to 0.5 meq. (9–10 mg.) per g. On a diet providing about 1500 mg. sodium, an uptake of 1–1.3 meq. per g. may be expected. We have found that 45 g. ammonium sulfonate resin, given in conjunction with a diet providing 400–500 mg. sodium and about 3000 mg. potassium per day, consistently reduces the urinary sodium to 1–2 meq. and the potassium to 5–6 meq. per day.[14] This indicates an adsorption of 0.4 meq. sodium, and 1.8 meq. of potassium, per g. of resin ingested. These data, although rather fragmentary, indicate the same order of efficiency for the two types of resins, and the laboratory and clinical findngs correlate quite well.

Another aspect of the problem of *in vivo* action concerns the influence of the ratio of sodium to potassium in the diet on the uptake of these two ions. It was thought earlier that the extra-fecal sodium and potassium resulting from resin ingestion would be in the same proportion as, and would not exceed the amounts in, the diet.[4] TABLE 2 shows some data pertinent to this point. They were obtained in the same way as in TABLE 1. If comparisons of action are made at the 10 per cent resin level, it is seen that the sodium uptake of the resin tends to increase with the sodium intake, while the total sodium-plus-potassium uptake remains almost constant, except on the very low salt diet. The ratio of (extra-fecal) sodium to potassium depends on the relative intakes of the two ions, but the relationship is not a direct one, nor does it even approach the 25 to 1 ratio of sodium to potassium which has been reported to exist in the intestinal tract of a fasting animal.[16] In the intestinal fluids of these animals during active digestion, the ratio of sodium to potassium was evidently much lower, perhaps 3 to 1.

TABLE 2

EFFECT OF COMPOSITION OF DIET ON THE IN VIVO UPTAKE OF SODIUM AND POTASSIUM BY SULFONIC RESIN. (WIN 3000)

Composition of diet*		% Resin in diet	No. of trials‡	*In vivo* uptake,§ meq./g., average		Total (Na + K)	Ratio Na/K Uptake
Na	K			Na	K		
2.6	3.6†	5	2	0.80	0.67	1.47	1.20
2.5	3.4†	10	2	0.45	0.50	0.95	0.90
9.2	11.1	5	5	0.55	0.37	0.92	1.49
9.2	10.5	10	5	0.78	0.60	1.38	1.30
9.2	9.9	15	5	0.53	0.60	1.13	0.88
12.6	17.6	10	2	0.85	0.52	1.37	1.63
28.2	17.2	10	7	1.04	0.36	1.40	2.90
28.2	27.5	10¶	10	1.05	0.43	1.48	2.45

* As meq. per 100 g. of food.

† This diet was prepared according to the formula of Chen and Freeman[15] for a salt free diet. The sodium and potassium found presumably originated in the brewer's yeast.

‡ Each "trial" represents the results in one pair of rats for a period of 5 or 6 days.

§ *i.e.*, excess fecal output over control animals receiving the same basal diet.

¶ The resin used in this case was Katonium,® a 75/25 mixture of Win 3000 (ammonium sulfonate resin) and potassium sulfonate resin. This mixture contributes an increment of 10 meq. potassium noted in the diet.

The results given in TABLE 2 demonstrate several other interesting points. From the data in line 2, an experiment involving a very low salt diet, it may be calculated that the sodium adsorbed by the resin was 237 per cent of the sodium content of the food ingested, and that the potassium uptake was 147 per cent of that present in the diet. These results may be attributed in part to low food consumption (it was one-third of normal) but they demonstrate directly, nevertheless, that it is possible for the resin to extract endogenous sodium and potassium from the digestive fluids.* The data in line 1 show that, at this very low level of mineral intake, 5 per cent resin in the diet is almost as efficient in sodium and potassium removal as is 10 per cent. In lines 3–5, data on an experiment involving moderate mineral intakes are shown. The interesting point to be noted here is that both 5 and 15 per cent intakes of resins are less efficient in cation removal on a per g. basis than is 10 per cent. One would expect the latter result, but not the former, since at the low level of resin intake more cations should be available for combination per g. of resin. In line 4, the sodium and potassium uptakes of the resin are somewhat less than the amounts provided in the diet, but, when one adds to these figures the amounts normally present in the feces (1 meq. sodium, 1.4 meq. potassium per 90 g. of basal diet consumed), the total fecal sodium becomes 96 per cent of the intake, and the total fecal potassium becomes 70 per cent of the intake.

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* Chen and Freeman[15] also observed this phenomenon. Their animals also consumed little food.

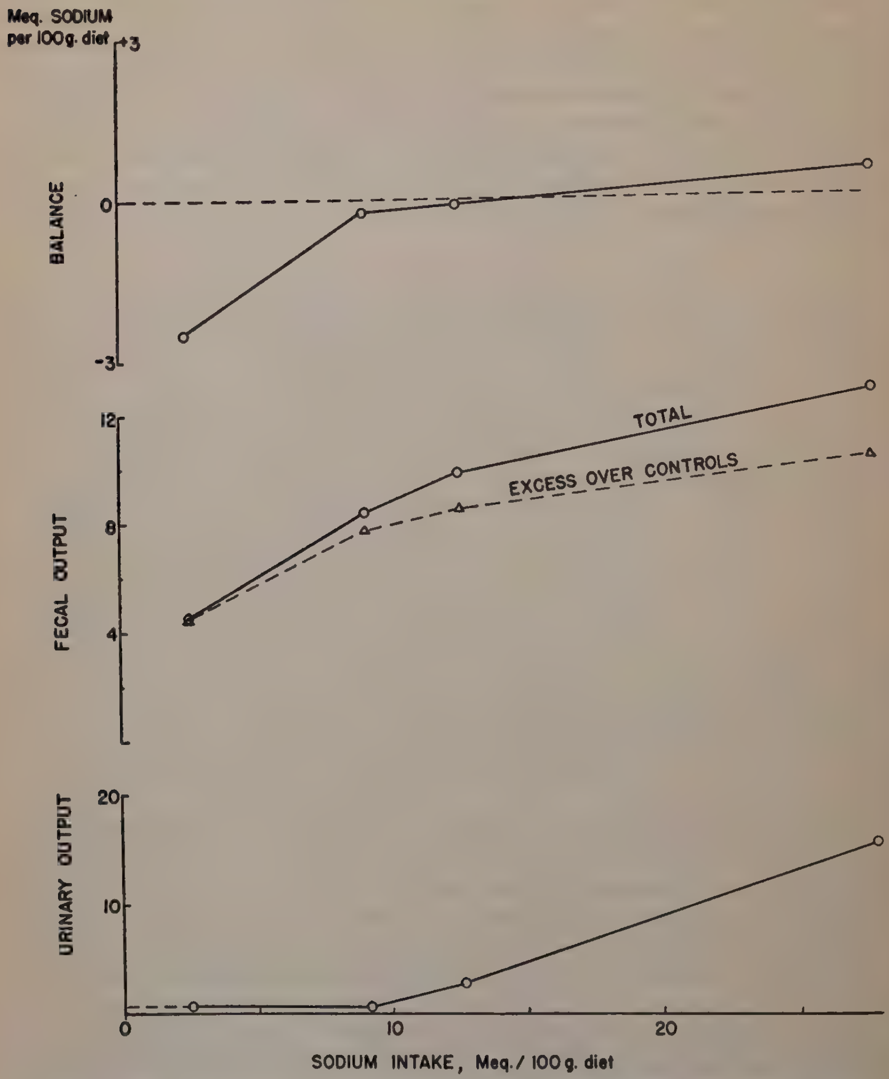

FIGURE 2. Effect of varying levels of sodium intake on urinary and fecal output of sodium and on external sodium balances, when 10 per cent ammonium sulfonate resin is included in the diet. Under fecal output "excess over controls" represents the difference between total fecal output of the animals receiving resin and of control animals receiving the same diet without resin.

In other words, it is actually possible, even at this level of sodium intake, to maintain the net sodium absorption of normal animals close to zero.

The entire sodium excretion pattern of the animals in lines 2, 4, 6 and 7 of TABLE 2 is shown graphically in FIGURE 2, and the following conclusions are indicated. Urinary sodium does not fall below a certain level (about 0.6 mg. per rat per day) even when sodium absorption is negligible, or negative. When the sodium intake exceeds 1 meq. per g. of resin ingested the urinary sodium begins to rise, and most of the excess above that level of intake appears in the urine. Fecal sodium probably does not become zero even if the intake is zero, but remains at a level about 3.5 meq. per 100 g. of diet ingested. Such a condition was not actually established in these experiments, but the data of Chen and Freeman support that view. Sodium balances are negative at the very low levels of intake, are just about zero at moderate levels of intake, and are slightly positive at the high levels of intake. The greatest efficiency of sodium removal is attained at an intake of 9 meq. per 100 g. of food; i.e., this was the highest level of intake at which urinary sodium was minimal.*

TABLE 3

WET AND DRY WEIGHTS OF STOOLS OF SUBJECTS INGESTING 45 g. RESIN DAILY. THE CONTROL AND MEDICATION PERIODS WERE SEVEN DAYS EACH.

	Subject	Control, Average	Sulfonic Resin		Carboxylic Resin	
			Average	Increase	Average	Increase
No. stools per day, average	A	1.0	1.0	0	1.43	0.43
	B	1.57	1.43	−0.14	2.00	0.43
				−0.07		0.43
Dry weight of stools, gm. average	A	30.3	51.1	20.8	65.4	35.1
	B	44.5	67.7	23.2	69.4	24.9
				22.0		30.0
Wet weight of stools, gm. average	A	116.7	141.2	24.5	209.0	92.3
	B	185.8	208.6	22.8	281.1	95.3
				23.6		93.8

As regards the removal of nutrient elements such as vitamins, or critical minerals other than sodium and potassium, it has been assumed[17] that the carboxylic type of resin is less likely to adsorb these substances and, if so, to be more specific for sodium. We have examined this point in some detail,[18] and have found no significant differences between the two types of resins as to the fecal outputs of calcium, magnesium, iron, amino acids, thiamine, or riboflavin. Growth experiments,[19] such as those cited earlier, support the finding that sulfonic resin permits an adequate absorption of all of the essential dietary substances. The four major metallic elements, however, seem to account for only 2.7 meq. per g., or 60 per cent of the total capacity of the sulfonic resin,[18] and since the adsorption of other substances is apparently negligible, we feel that the remaining capacity must be assigned to ammonium and hydrogen ions. The quantitative aspects of this problem have been discussed elsewhere[18] and need not be repeated here.

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* If these findings were transferable to man, it would mean that an intake of 45 meq. sodium for 45 gm. of resin should provide the condition of "greatest efficiency" as defined above.

As we have seen, resin therapy necessitates daily doses of about 45 g., which in turn means an increase in the solid content of the feces of about 100 per cent. Thus a comparison between the two types of resin with respect to laxative or constipative properties is of considerable interest. Data shown in TABLE 3 indicate that the sulfonic resin causes only one-fourth the bulking and swelling of the carboxylic. Actually the carboxylic resin swells almost as much as one of its non-crosslinked chemical analogs, methylcellulose, a product which is actually used as a bulk laxative.[20] Yet in the case of products used as bulk laxatives one selects soft emollient colloids; whereas the grittiness of these resins makes the added bulk not therapeutically desirable, therefore the sulfonic type resin has a clear advantage in this respect since its bulking effect is so much less. The greater number and bulk of stools from the carboxylic resin produces a greater sense of fullness and bloating in the patient. Perhaps the most careful tabulation of gastrointestinal symptoms resulting from resin administration is in the paper by Callahan *et al.*,[21] who studied 22 patients on each of the two types of resin. They found a greater incidence of undesirable symptoms in the patients receiving the carboxylic resin, with nausea and anorexia as the outstanding complaints. Constipation was noted in six of the carboxylic resin subjects and in five of the sulfonic resin subjects. These authors reported 12 "successful" treatments in each of the 22 cases. The sulfonic resin was found to cause hypocalcemia and hypopotassemia in some patients, presumably because the product used was a straight ammonium resin. We have not observed hypocalcemia with either type of resin, nor have we observed any tendency to constipation or other serious gastrointestinal symptoms in patients receiving 15 g. sulfonic resin 3 times daily and 2 Caroid and Bile Salt tablets (American Ferment Co.) twice daily. Even if this incidence of adverse gastrointestinal effects is generally confirmed,[22] other things being equal, the sulfonic resin should become the treatment of choice. It is significant that pharmaceutical houses in both Great Britain and Germany have preferred to introduce the sulfonic type for sodium removal.

A combination of carboxylic resin with basic anion exchange resin has been introduced on the supposition that anions and cations can be removed simultaneously, thus effecting a partial desalting of the food. Our animal data[23] indicate that the incorporation of anion exchange resins does not increase the sodium removal efficiency of carboxylic resin; rather it increases the bulk at the expense of cation exchange resin which might otherwise have been employed to divert additional amounts of sodium ion, and does not remove important amounts of anions.

Summary

Both laboratory and clinical investigations indicate that sulfonic and carboxylic ion exchange resins are capable of removing from the food 0.4–0.5 meq. (10 mg.) sodium per g. on a low sodium daily diet and from 0.9 to 1.2 meq. (20–27 mg.) on a medium or high sodium intake. The last figure of 1.2 meq. of sodium corresponds to a daily diversion of 3.2 g. sodium chloride

for a 45 g. daily resin dose. Laboratory data indicate a superiority with respect to capacity for the sulfonic resin. Clinical data are not comprehensive enough as yet to settle this point finally, but the incidence of undesirable side-effects such as bulking and unpleasant gastrointestinal symptoms which are likely to occur with the carboxylic type, give a strong indication that the use of sulfonic resin will become the treatment of choice.

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CLINICAL EXPERIENCE WITH MIXTURES OF ANION AND CATION EXCHANGE RESINS

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Our initial clinical trial of ion exchange resin for the removal of sodium from the gastrointestinal tract was made with a sulfonic acid type resin in the hydrogen form. Administration of this material resulted in complaints of a burning sensation in the mouth and throat and epigastric discomfort.

The occurrence of these unpleasant symptoms led to the trial of a carboxylic type resin. The administration of 60 grams of carboxylic resin per day in the hydrogen form was well tolerated, but after 8 days the concentration of potassium in the plasma had decreased from 4.3 mEq. to 2.9 mEq. per liter in a normal subject and clinical signs of potassium deficiency were present. We found this deficiency could be avoided by the administration of potassium chloride. Another way of supplying potassium was the administration of a resin which had been saturated with this element.[1] In 0.05 N hydrochloric acid the hydrogen ion displaced potassium from the carboxylic type resin. Therefore, in the stomach almost all of the potassium would be released from the resin. If all of the carboxylic resin needed to remove from 30 to 60 mEq. of sodium per day were to be administered in the potassium form, the quantity of potassium which would be released therefrom and absorbed by the intestines might exceed the excretory capacity of the kidney.

In two of our patients with moderate impairment of renal function, administration of 30 grams of potassium resin per day resulted in a gradual increase in the concentration of potassium in the plasma to values above normal. By clinical trial of mixtures of carboxylic resin in the hydrogen and potassium forms, it was established that, when approximately one-third of the total dose was given as a potassium resin, urinary concentration of this cation was maintained at approximately the pretreatment level. The proportion of potassium used in this mixture is in agreement with the amount recommended by McChesney.[2] This investigator has recently reported that the optimal potassium content of cation resin is 1 to 2 mEq. potassium per gram of resin. Our studies have revealed that there was some loss in efficiency in the removal of sodium when a potassium form of resin was incorporated in the mixture. For general clinical application, however, this disadvantage is offset by the greater convenience of not having to ingest supplementary potassium.

In several patients with moderate impairment of renal function the daily administration of 40 grams of carboxylic resin in the hydrogen form and 20 grams of this resin as the potassium salt caused a gradual rise in the concentration of the chloride in the plasma and a decrease in the carbon dioxide content. The limited ability of the renal mechanism to synthesize ammonia, as first pointed out by Kahn and Emerson,[1] is the cause for this abnormality in the anion pattern of extracellular fluid.

Analysis of feces collected during administration of cation exchange resins revealed that the excretion of phosphate by way of the bowel had decreased.

During the same period a rise in urinary phosphate was noted. These observations indicated that the anion excretory load of the kidney was increased and the additional phosphate contributed to the need for synthesis of ammonia by the cells of the renal tubules.

Use of anion exchange resin in combination with a cation exchanger. A desire to reduce the anions excreted in the urine caused us to investigate the use of a polyamine type anion exchange resin. The preparation known as XE-58 was selected because it was a weak base type of exchanger and had been used in clinical medicine. The action of this anion exchange resin in the presence of carboxylic cation exchange resin was first studied in a series of *in vitro* experiments. Two grams of various mixtures of anion and cation resins were exposed for 1 hour to 100 ml. of a buffered phosphate solution at pH 6.7, with continuous stirring. The concentration of electrolytes in this solution was made to approximate that found in the human intestine. In these *in vitro* experiments a portion of the cation exchanger was in its potassium form, because, as has already been shown, it was desirable to incorporate this type of exchanger in a preparation for clinical use.

TABLE 1

IN VITRO STUDY OF MIXTURES OF CATION AND ANION EXCHANGE RESIN

Resin mixture, per cent		Final pH of medium	Change in electrolyte concentration expressed in mEq./gm. of resin mixture			
Cation*	Anion†		Na$^+$	K$^+$	Cl$^-$	PO$_4^{---}$
100	0	5.75	−1.13	+1.20	0	0
95	5	5.90	−1.25	+1.13	0	−0.21
85	15	6.12	−1.61	+0.97	−0.17	−0.61
75	25	6.25	−1.32	+0.84	−0.37	−0.84
65	35	6.45	−1.32	+0.70	−0.52	−1.03
0	100	8.71	0	0	−0.31	−0.12

* Cation resin = 2 parts carboxylic type resin in hydrogen cycle (XE-64), 1 part potassium cycle XE-88.

† Anion resin = polyamine type of exchanger (XE-58).

Procedure: Two grams of each resin mixture was exposed for one hour, with continuous stirring, to 100 cc. of a solution buffered to pH 6.7 containing electrolytes in the following concentration: sodium 130 mEq./l., potassium 10 mEq./l., chloride 120 mEq./l., phosphate 40 mEq./l.

When anion resin alone was placed in the solution that simulated intestinal fluid some chloride and phosphate ions were removed, but a greater uptake of these anions occurred when a mixture of cation and anion resin was added to the test solution. As more and more of the anion resin was incorporated in the mixture, chloride and phosphate removed per gram of resin increased.

The presence of anion resin also improved the efficiency of the cation exchanger (TABLE 1). As the percentage of anion resin in the mixture was raised, the pH of the solution containing electrolytes and resins underwent less change than when either cation or anion resin alone was placed in this environment. The results of these experiments indicated that the optimal anion concentration for maximum sodium removal was between 5 per cent and 15 per cent.

For all subsequent studies a mixture containing 12 per cent of the polyamine anion resin and 59 per cent carboxylic resin in the hydrogen form and 29 per cent as the potassium salt of the latter resin was employed. As is immediately obvious, many features of an *in vitro* study are in no way comparable to the phenomena occurring in the human gastrointestinal tract. Also, in our experience, the rat has not proved to be a completely satisfactory test subject for determining the efficiency of sodium-removing resins. In our laboratory, the capacity of the resin to remove sodium as observed in rats has often been less than one-third as great as that found by clinical trial in human subjects.[3] For this reason, the effect of the administration of mixtures of anion and cation resins was subsequently investigated in patients with heart disease who exhibited no clinical signs of failure at the time of the experiment.

These studies were conducted in a special ward, equipped for metabolic procedures and staffed with trained personnel including a full time nutritionist. The sodium content of all food was determined in our own laboratory by analysis of sample specimens. The same menu was used during each test. All servings were weighed and correction was made for uneaten food. Specimens of feces were collected over a period of three days. The beginning of the test and its end were marked by carmine dye.

TABLE 2

EFFECT OF ANION-CATION EXCHANGE MIXTURES ON THE FECAL EXCRETION OF SODIUM AND PHOSPHATE AND ON THE URINARY EXCRETION OF PHOSPHATE

Resin mixture gm./day			Total dose resin gm./day	Fecal excretion		Sodium Removed mEq./gm. resin	Urinary excretion phosphate mM./day
Cation		Anion		Phosphate mM./day	Sodium mEq./day		
K^+	H^+						
—	—	—	0	22	5	—	24
34	17	—	51	11	28	0.5	36
30	15	6	51	17	57	1.1	30
18	15	18	51	21	45	0.9	24
12	15	24	51	23	39	0.8	23
12	15	—	27	11	10	0.4	33

After a change of diet or resin dosage, collection of feces was not begun until urinary excretion of sodium and potassium had been constant for several days. The effect of the use of anion exchange resin with the cation resin on the fecal excretion of sodium and phosphate and the urinary excretion of phosphate was observed by administering various mixtures of these resins to the same patient (TABLE 2).

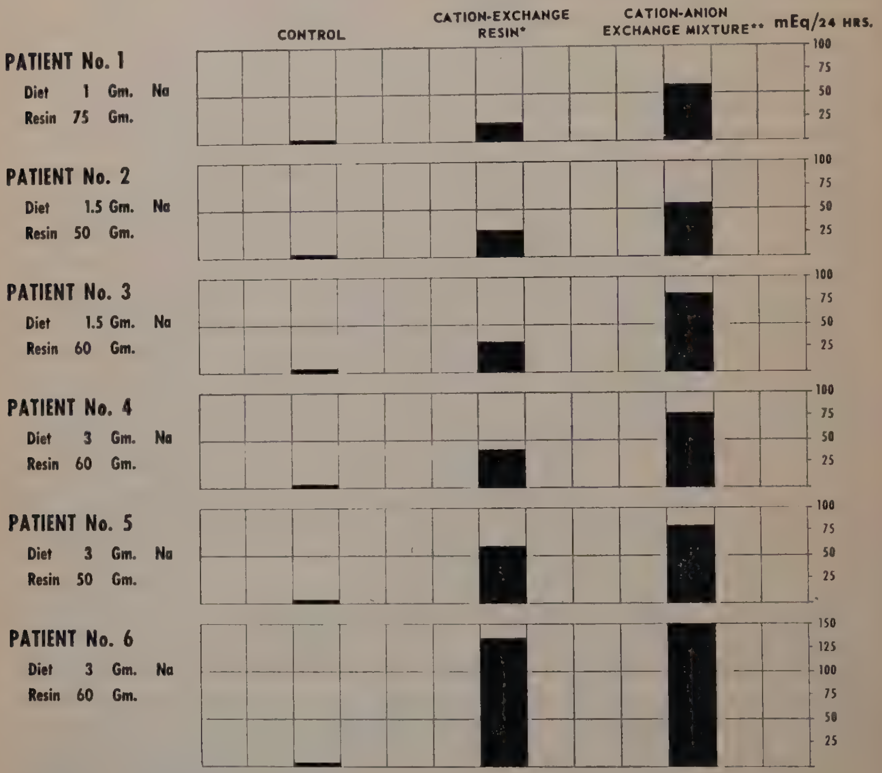
After a control period, the subject was given a carboxylic type of cation resin alone. During the next three periods, mixtures containing varying amounts of cation exchange resins were administered. The total dose of resin in grams per day was the same for each of these tests and the amount of carboxylic resin in the potassium salt was constant in all preparations that contained anion resins. The greatest decline in phosphate in the feces occurred when cation resin alone was administered. As more and more anion exchanger was added the fecal excretion of phosphate came to approximate that of the control period. The resin combination was most efficient for removal of sodium when it contained 12 per cent or 6 grams of anion exchanger in 51 grams of the mixture. The sodium-removing capacity decreased slightly with the addition of a greater percentage of anion resin. Changes in urinary excretion of phosphate bore an inverse relationship to the concentration of this anion in the feces.

In the final period of this study, the same quantity of the carboxylic resin was given as had been used in the mixture containing 4 grams of the anion exchanger. As in the intial experiment, phosphate excretion in the feces was again sharply depressed as was that of sodium. These results indicate that the decrease in phosphate excretion was not the result of a diminution in the amount of cation resin employed and confirmed the findings obtained from *in vitro* studies. Although the change in fecal excretion of phosphate when expressed in millimols per day is minimal, the decrease in urinary excretion of phosphate achieved by the use of anion resin may be of significance when resins are given continuously to patients with a limited ability to synthesize ammonia. Furthermore, the incorporation of anion exchange resin enhanced the sodium-removing capacity of the cation exchanger. Further evidence of the increased efficiency gained by use of the combination of anion and cation exchange resins was obtained by comparing, in 6 patients, the sodium-removing capacity of the resin mixture containing 12 per cent anion exchanger (FIGURE 1). In each patient, the fecal excretion of sodium was measured during a control period, during the administration of cation resin alone, and during the administration of the mixture of anion-cation resins. One-third of the cation exchanger was in the form of its potassium salt. The sodium in the diet varied from 1 gram to 3 grams per day, and the total amount of resin administered ranged from 50 grams to 75 grams per day.

It should be noted that when the mixture contained anion resin the patient received only 88 per cent as much cation exchanger as when the latter type of resin was given alone. The order of the experiments was not always as shown in the chart. Some persons received the mixture of anion-cation resin during the first period and in others the administration of resins preceded the control period. It is apparent that, in all experiments, the amount of sodium excreted in the feces per 24 hours was greater when 12 per cent anion resin was incorporated in the mixture than when cation exchanger alone was given. As the sodium in the diet was increased, there was a definite improvement in the efficiency of the resin. It should also be noted that there was some variation from individual to individual when the same diet and resin dosage was employed.

Resin in congestive heart failure and cirrhosis. We have had an opportunity to measure the fecal excretion of sodium and potassium and the urinary excretion of sodium, chloride, and ammonia 16 times in patients with cirrhosis of the liver or congestive heart failure who were receiving the resin mixture containing 12 per cent anion exchanger (TABLE 3). These studies were conducted on the special ward and the procedure as previously described was employed for collection of feces. The minimum efficiency for sodium removal was 0.6 mEq. per gram of resin mixture, and the greatest uptake of sodium in any of these studies was 1.7 mEq. per gram of resin. In ten periods of observation the total sodium excreted in the urine and feces exceeded the quantity of this substance supplied by the diet. In Experiment No. 7, this loss surpassed the intake of sodium by 34 mEq. per day.

These data reveal that a negative sodium balance can be produced by the administration of ion exchange resins, but the amount of resin requred and the degree of dietary restriction necessary may vary from patient to patient. Furthermore, we have observed that the efficiency of the resin mixture may vary from time to time in the same patient. In all but one instance the amount of potassium in the feces exceeded the concentration of sodium. In one person (Experiment No. 8), slightly more than three times as much potassium as sodium appeared in the feces.



*33⅓% Potassium, 66⅔% Hydrogen
**29% Potassium, 59% Hydrogen, 12% Anion Exchange Resin

FIGURE 1. Comparison of fecal excretion of sodium during the administration of cation exchange resin and of the cation-anion exchange mixture. In each patient the same menu and quantity of food was employed during the control period and the two periods of resin administration.

Resins and dietary restriction of sodium in the treatment of hypertension. From our experience with ion exchange resins in the treatment of congestive heart failure and cirrhosis it became evident that they might be used as an adjunct to dietary restriction of sodium in the treatment of essential hypertension. Corcoran, Taylor, and Page[4] have estimated that the minimum limitation of sodium required to lower blood pressure is 0.5 gram per day, and this restriction must be continued indefinitely. The rice-fruit diet, as popularized by Kempner[5] provided a satisfactory method for obtaining the needed limitation, but many persons were unable to tolerate the monotony of this regimen.

We proposed to use both diet control and sodium removal by ion exchange resins in the treatment of essential hypertension. Both therapeutic measures were employed in order to shorten the time needed to lower the blood pressure or to reduce the pressure in patients who had failed to respond to dietary restriction alone. During the course of our study, two reports (Groff,[6] and Gill and Duncan[7]) have been published describing favorable results with the administration of cation exchange resins along with a low sodium diet.

TABLE 3

EFFECT OF ION EXCHANGE RESINS ON FECAL AND URINARY EXCRETION OF SODIUM AND POTASSIUM

Exp. no.	Diagnosis	Dietary sodium mEq./day	Resin dosage gm./day	Urinary excretion mEq./24 hr.			Fecal excretion mEq./24 hr.		Feces $\frac{K}{Na}$	Feces Na^+ mEq./gm. resin	Daily sodium balance‡ mEq.
				Na^+	Cl^-	NH_4^+	Na^+	K^+			
1	CHF*	40	68	14	57	80	44	89	2.0	0.6	−18
2	CIRR†	40	83	2	60	127	58	141	2.4	0.7	−20
3	CHF	65	24	35	49		37	41	1.1	1.5	−7
4	CHF	65	51	9	64	109	57	123	2.2	1.1	−1
5	CHF	65	68	33	60		60	136	2.3	0.9	−28
6	CIRR	65	48	18	74		32	37	1.2	0.7	+15
7	CIRR	65	80	2.2	57	50	97	111	1.1	1.2	−34
8	CIRR	65	96	2.5	73	109	56	177	3.2	0.6	+6
9	CIRR	65	68	20	96	96	48	89	1.9	0.7	−3
10	CIRR	130	24	68	92	40	17	18	1.1	0.7	+45
11	CHF	114	48	37	103	136	81	97	1.2	1.7	−4
12	CHF	114	48	40	105	136	56	73	1.3	1.2	+18
13	CHF	130	51	47	133		84	62	0.7	1.6	−1
14	CHF	130	68	40	92		83	89	1.1	1.2	+7
15	CHF	130	68	52	116	105	77	139	1.8	1.1	+1
16	CIRR	130	51	63	118	93	79	84	1.1	1.5	−12

* Congestive heart failure.
† Cirrhosis of the liver.
‡ Dietary intake of sodium minus sum of fecal and urinary excretion.

Eighteen patients considered to have hypertension of a severe degree were selected from a group of individuals under observation in our clinical laboratory. They had all been hospitalized on previous occasions for the clinical trial of new therapeutic agents and were accustomed to the routine of our ward. In the majority of these persons the course of the disease had been well documented. With one exception treatment was begun on the special hospital ward. At the time of admission all medication that could conceivably influence arterial pressure was discontinued. During hosptialization all patients were encouraged to remain ambulatory, although each person rested in bed for at least 30 minutes before the blood pressure was taken. The pressure was recorded twice daily by the same physician. The determination was repeated by another member of our staff whenever any change in the level of arterial pressure occurred.

In 12 patients, it was possible to arrange for a period of observation in the hospital prior to treatment. In these persons, treatment was begun with a diet which supplied 500 mg. of sodium and 48 grams of the cation-anion resin mixture per day. Four patients began dietary restriction and the resin mixture immediately upon admission to the hospital. In these instances, a control period in which the arterial pressure was allowed to rise to pretreatment level was instituted after the initial response to therapy had been obtained.

In three individuals, when the maximum benefit of a combination of a 500-mg. sodium diet and 48 grams of resin appeared to have been reached, the diet was liberalized by increasing the daily allotment of sodium to 1.5 grams and giving an additional quantity of resin. When the patient received 64 grams or more of the resin mixture daily the urinary excretion of sodium remained at approximately the same concentration as had been present during the time the patient was receiving the 500 mg. sodium diet and 48 grams of resin.

In four subjects, treatment was begun with the 1.5 gram sodium diet and sufficient resin to maintain a very low concentration of sodium in the urine. One individual in this group was treated entirely as an outpatient. The diets used were calculated on the basis of the sodium content of samples of food submitted for analysis in our own laboratory. The 500 mg. sodium diet supplied approximately 300 grams of carbohydrate, 68 grams of protein, and 72 grams of fat which provided about 2400 calories. The 1.5 gram diet consisted of 295 grams of carbohydrate, 86 grams of protein, and 102 grams of fat, yielding approximately 2900 calories. The results obtained by dietary restriction and concomitant administration of resin are summarized in TABLE 4. In 13 of 18 patients, there was a definite lowering of blood pressure. In 11 this reduction in arterial pressure was considered to be clinically significant. In eight of these patients the pressure was restored to a normal range (less than 150/90 mm. Hg).

Despite the restriction of sodium in the diet to 0.5 gram per day and the administration of 48 grams of resin, in five persons the average blood pressure was not altered. In two instances, sodium in the urine was not decreased in these subjects as much as it had been in patients in whom the lowering of pressure occurred. In the remaining three individuals, however, the urinary concentration of sodium was not more than it had been in patients in whom satisfactory results were obtained. The average decline in systolic pressure in the 13 persons in whom the lowering of arterial pressure was considered definite was 48 mm. Hg, with a range of 15 to 72 mm. Hg. The average fall for the diastolic pressure was 20 mm. Hg, with a range of 15 to 32 mm. Hg. The maximum lowering of pressure occurred between the second and sixth week and a significant drop was noted in less than four weeks in patients receiving a 500 mg. sodium diet plus 48 grams of resin.

Balance studies were conducted on 3 patients who had received a 500 mg.

TABLE 4

RESULTS OF THE ADMINISTRATION OF ION EXCHANGE RESINS AND RESTRICTION OF DIETARY SODIUM IN HYPERTENSION

Patient	Age	Known duration of hypertension (years)	Dietary sodium gm./day	Resin dosage gm./day	Blood pressure mm.Hg Average 14 pressures (1 week)		Week of treatment for lowest blood pressure	Observations during week of maximum lowering of blood pressure				Classification of therapeutic response
					Control	Lowest with resin		Average daily urine sodium, mEq.	Systolic pressure, mm. Hg	Change in Diastolic pressure, mm. Hg	Body weight, kg.	
M. B.	49	9	0.5	48	194/104	136/82	5	2.0	−58	−22	−1.8	N
			1.5	64		131/74	1*	2.0	−63	−30	−1.3	
E. B.	45	8	0.5	50	176/94	129/81	2	3.3	−47	−13	−1.4	N
L. C.	48	12	0.5	48	180/96	148/82	5	3.0	−32	−14	−3.4	N
			1.5	64		140/80	2*	2.7	−40	−16	−3.8	
R. F.	36	5	0.5	48	170/110	116/79	5	1.8	−54	−31	−7.0	N
P. F.	55	19	0.5–1.5	48–72	173/92	148/83	5	4.2	−19	−12	+0.4	N
H. P.	30	unknown	0.5–1.0	48	178/104	124/85	3	2.8	−54	−19	−1.8	N
M. T.	49	8	1.5	64	199/100	150/85	4	5.0	−49	−15	+1.5	N
			1.5	96		127/80	3†	1.7	−72	−20	−3.0	
W. W.§	33	unknown	1.5	64–80	188/122	125/90	8	4.0	−63	−32	+1.0	N
W. C.	38	8	0.5	48	203/131	162/112	3	3.7	−41	−19	−4.9	+
B. L.	64	16	0.5	48	208/116	164/90	6	2.2	−44	−26	−4.9	+
E. D.	41	7	0.5	48	215/109	163/93	4	3.0	−52	−16	0.0	+
J. B.	55	2	0.5	48	227/122	212/100	3	3.9	−15	−22	−2.7	±
V. P.	36	6	1.5	84	213/129	191/116	4	3.8	−22	−13	−1.4	±
F. S.	51	10	1.5	68	221/109	194/108	5	2.7	−27	−1	−1.8	−
H. W.	50	5	1.5	68–80	186/99	199/100	5	7.3	+13	+1	−0.7	−
			0.5	48		178/98	5‡	4.2	−8	−1	−1.3	
G. T.	28	5	0.5	48	189/112	192/116	6	7.0	+3	+4	−3.2	−
M. M.	32	unknown	0.5	48	223/133	213/121	3	3.7	−19	−12	−0.4	−
H. D.	33	9	0.5	48	185/125	177/119	5	3.1	−8	−6	−4.9	−

* Weeks after diet and resin increased.
† Weeks after readmission to hospital.
‡ Weeks after diet and resin decreased.
§ Treated as an outpatient.
N Pressure reduced to normal values (150/90).
+ Definite lowering of pressure, but not to normal values.
± Equivocal result.
− Negative result.

sodium diet and 48 grams of the cation-anion resin mixture for 10 to 30 days. In these subjects, the excretion of sodium in the urine and feces exceeded the quantity supplied in the diet by 18 to 24 mEq. per day. In these patients, each gram of the resin mixture removed 0.7 to 0.8 mEq. of sodium. When similar studies were conducted on patients who received 1.5 grams of sodium in the diet and 64 grams of the resin mixture, the excretion of sodium in the urine and feces was not more than 70 mEq. per day. In these persons, the total loss of sodium rarely surpassed the amount supplied in the diet.

In patients on the 500 mg. sodium diet, the average loss of body weight which occurred between the beginning of the administration of 48 grams of resin per day and the week in which the maximum lowering of arterial pressure occurred was 3.2 kg. The minimum weight loss in this group was 0.4 kg. during five weeks of treatment and the maximum loss was 7 kg. The latter patient was an individual who was permitted to continue his work in the chemistry laboratory during the time he was receiving the 500 mg. sodium diet and 48 grams of the resin mixture. W. W., TABLE 4, who was treated as an outpatient on a 1.5 gram sodium diet and 64 grams of resin, gained 1 kg. during 8 weeks of treatment. During this time, the blood pressure had been reduced from an average of 188/122 mm. Hg to 125/90 mm. Hg. During the week of the maximum lowering of the blood pressure, the quantity of sodium in a 24-hour urine specimen averaged 4 mEq.

TABLE 5

EFFECT OF DIETARY RESTRICTION AND RESIN ADMINISTRATION ON PLASMA ELECTROLYTES

	No. of patients	No. of determinations	Mean mEq./l.	Range mEq./l.	
Control Period					
Sodium	16	38	141.7	139.1–143.5	
Potassium	16	38	3.99	3.53–4.61	
Chloride	17	39	103.6	93.0–107.3	
Carbon dioxide content	17	39	29.6	25.5–33.0	
Period of Resin Administration*					
					Change mEq./l.
Sodium	17	37	140.1	133.2–143.5	−1.6
Potassium	17	37	4.05	3.29–4.54	+0.06
Chloride	17	37	107.7	100.0–118.0	+4.1
Carbon dioxide content	17	37	23.7	17.0–28.4	−5.9

* Results obtained during week of lowest average blood pressure.

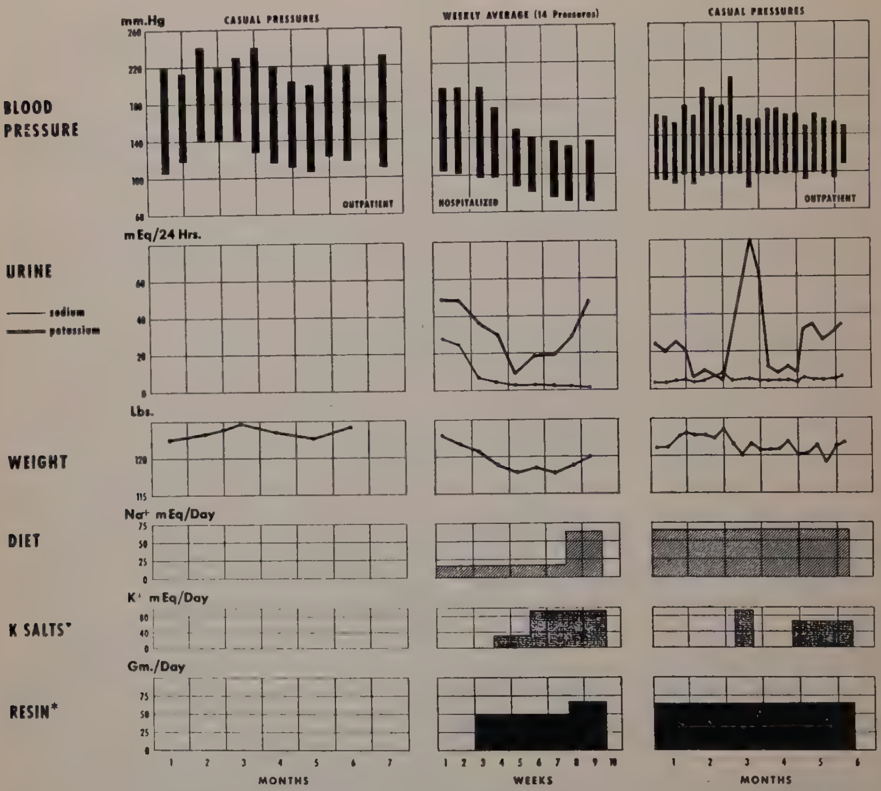
Changes in the sodium, potassium, chloride, and carbon dioxide content of the plasma are summarized in TABLE 5. In patient M. B., TABLE 4, the sodium in the plasma was found to be 133 mEq. per liter. This patient was on a 500 mg. sodium diet and was receiving 48 grams of the resin mixture per day. The sodium in the diet was increased to 1.5 grams and the resin dosage was raised to 64 grams per day. Under these conditions, the concentration of sodium in the plasma quickly returned to 141 mEq. per liter. In all other patients, there were no significant changes in the concentration of electrolytes in the plasma during administration of resin when the patients were on a diet restricted in sodium.

Use of potassium supplement. A decline in the potassium content of the plasma was avoided by the oral administration of a mixture of organic potassium salts which supplemented the potassium supplied by the carboxylic resin. All patients receiving 48 grams of the cation-anion resin mixture were given orally at least 45 mEq. of added potassium and, in some instances, as much as 120 mEq. of potassium per day. A preparation composed of equal parts of potassium acetate, citrate, and bicarbonate was used. The anions supplied were thus metabolized, hence an increase in the excretory load of the kidney was avoided. When the potassium was administered between doses of resin there was no evidence that the removal of sodium declined. The occurrence of subjective symptoms such as anorexia, lethargy, constipation, and weakness signified an impending potassium deficiency. If the potassium supplied by the resin and diet was not supplemented, nausea, extreme weakness, and changes in the electrocardiogram soon appeared. In some patients who exhibited untoward symptoms before the administration of potassium was begun, the effect of a single dose of 30 mEq. of potassium was striking.

An example of the results obtained with dietary restriction of sodium and the use of the cation-anion resin mixture is illustrated in FIGURE 2. This patient was known to have had hypertension for 9 years. During treatment in the hospital, the average blood pressure was reduced to normal. It should be noted that the sodium in the urine did not rise nor did the arterial pressure increase when dietary sodium was liberalized to 1.5 grams per day and the dosage of resin was increased from 48 to 64 grams per day. The casual blood pressure readings obtained during treatment as an outpatient were higher than those obtained during hospitalization. The arterial pressure was definitely lower after release from the hospital, however, than it had been in the pretreatment period. In this patient, oral administration of the organic potassium salts also did not change the concentration of sodium in the urine, but did cause a rise in the urinary excretion of potassium. The use of a cation-anion exchange resin mixture along with a restriction of dietary sodium to 500 mg. per day may be expected to expedite the treatment of essential hypertension.

Watkin and his associates[8] have pointed out that the amount of sodium that can be permitted in the diet while maintaining a reduced level of arterial pressure is so small that it has not been convincingly demonstrated that sufficient sodium depletion can be achieved except by use of the rice diet or by very severe restriction of food intake. Corcoran, Taylor, and Page[4] have concluded that outpatient low sodium diet therapy is impractical and difficult. The use of ion exchange resins with moderate dietary restriction appears to have overcome these difficulties.

In the treatment of essential hypertension with ion exchange resins and dietary restriction of sodium, the determination of sodium and potassium in the urine is desirable. This information offers a measure for the effectiveness of dietary control and is a reliable means of detecting an impending potassium deficiency. It should be emphasized that potassium supplementation should be instituted as soon as clinical signs of a deficiency appear. It is not necessary to wait for laboratory findings for confirmation.



▼ Mixture of potassium acetate, citrate, and bicarbonate

* Mixture of 59 per cent carboxylic resin in hydrogen form and 12 per cent potassium salt of this resin with 29 per cent polyamine anion resin

FIGURE 2. The changes in blood pressure, urinary excretion of sodium and potassium, and the body weight in a female with sustained hypertension treated by dietary restriction of sodium and administration of cation-anion resin mixture. The effect of organic potassium salts as a supplement to potassium in food and resin is also illustrated.

Comments. A preparation containing 59 per cent carboxylic resin in the hydrogen form, 29 per cent of the same resin as its potassium salt, and 12 per cent as a polyamine anion exchanger has been given to 50 patients with severe congestive heart failure, to 18 with cirrhosis and ascites, and to 18 persons with essential hypertension. Six of these patients have taken 30 to 60 grams of this resin mixture daily for more than two years. In these persons no clinical signs of calcium deficiency or impairment of nutrition have as yet been observed. In eight, gastrointestinal disturbances were of sufficient severity to interfere with the continued administration of the resins. Constipation has been the most frequent undesirable side effect, having been observed in about one-third of the patients in our study. The use of supplementary potassium has eliminated this untoward symptom in some instances, and in others an increase in the fluid intake has helped avoid dehydration of the resin in the lower bowel. A few patients have required methylcellulose, mineral oil, or some other laxative drug to facilitate elimination of the resin from the intestine. Diarrhea has occurred in a few instances. It was necessary for two patients to discontinue the use of the resin mixture.

During hospitalization, all patients with severe congestive heart failure who previously required severe dietary restriction and repeated injections of mercurial diuretics as often as twice each week, by the use of cation-anion resin mixture have been kept in an edema-free state and have been permitted an increase in their allotment of dietary sodium. Two patients have required an occasional injection of the mercurial after their release from the hospital.

In several instances, the resins have contributed to the rehabilitation of the cardiac patient. Frequent visits to the outpatient clinic have been avoided and repeated episodes of congestive failure have been eliminated. The diet has been liberalized in nearly all cases. These advantages have enabled the patient to hold a job within the range of his physical capacity.

In some patients with congestive failure, satisfactory results have been obtained by the *intermittent* administration of the cation-anion resin mixture. The resins are given for four consecutive days and omitted for three days. The recurrence of edema may be avoided by this regimen, but it is not suitable for the treatment of severe failure or hypertension.

Despite the presence of an anion exchange resin we have noted an increase in the concentration of chloride in the plasma, especially in persons with renal impairment. There has been a concomitant decrease in the carbon dioxide content. In the treatment of edema associated with congestive failure, an increase in the serum chloride appears to be desirable. In some persons who have been treated with organic mercurial in an injudicious manner, the administration of ion exchange resins has restored responsiveness to this medication.

Limited experience with the use of resin mixtures in the treatment of patients with cirrhosis of the liver, forming ascites, has revealed that usually larger amounts of resin are required despite a more severe dietary limitation imposed upon these patients. It is our opinion that ion exchange resins have proved to be a valuable addition to our therapeutic armamentarium for the control of edema and the treatment of hypertension.

New types of ion exchange resins are now under study in our laboratory. It is possible that a more efficient preparation than any now available may be developed. When the amount of resin needed to control edema is reduced, this form of therapy will receive wide acceptance in the practice of medicine.

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CHANGES IN FECAL AND SERUM CONSTITUENTS DURING INGESTION OF CATION AND ANION EXCHANGERS*

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The synthetic cation exchange resins have proved to be a useful adjunct in the prevention or delivery of edema,[1-26] in the treatment of potassium intoxication,[27] and of hypertension.[28-31] In the course of the clinical trails with carboxylic or sulfonic cation exchangers cited above, it has become apparent that the increase in fecal sodium per gram of resin is rather low, that wide liberalization of diets with respect to sodium content is usually not possible, that potassium and other ion deficits develop with prolonged therapy, and that undesirable degrees of acidosis may appear in patients with renal disorders. In the hope of further defining and perhaps circumventing some of these limitations, we have studied the effects of adrenocorticotrophic hormone or cortisone, sulfonic resins, ethylene diamine tetraacetic acid, or alkylene polyamine anion exchangers used in conjunction with carboxylic resins. A summary of the changes to be expected, when various forms of carboxylic type exchangers are used alone in children or adults, precedes the presentation of the results obtained with these various combinations. To obtain as large groups as possible for comparison purposes, published[32-40] and unpublished data from this laboratory have been pooled without distinction as to the sodium content of the diet or the nature of the illness which resulted in hospitalization.

From TABLE 1 it is readily apparent that the hydrogen, ammonium, and potassium forms of this particular carboxylic exchanger† augment the fecal excretion of sodium and of potassium to about the same degree, whereas the calcium form is less effective in increasing the loss of potassium via this route. This finding presumably reflects the greater affinity of the resin for divalent cations but this does not explain of course the continued significant excretion of sodium in this group of studies when patients received the calcium form of the exchanger. The large output of sodium during sodium form resin administration indicates that in this limited number of experiments the resin either failed to release this cation or interfered with its assimilation following displacement from the exchanger.

The summary (see TABLE 2) which follows, establishes that neither cortisone nor adrenocorticotrophic hormone therapy interferes with the ability of this resin mixture to increase to a moderate degree the fecal excretion of sodium. On the other hand, the potassium data are compatible with a resin induced rise in the output of this electrolyte. If this is indeed a pharmacological action of these hormones, it is quite in line with their demonstrated effect upon renal losses of potassium.

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The data presented in the first two tables illustrate the statement that these types of exchangers, irrespective of their form, possess a relatively low efficiency in altering the excretion of univalent cations in feces. Since the ordinary daily diet frequently contains a total of several hundred milli-equivalents of sodium and potassium, it is apparent that clinically significant interference with sodium absorption would necessitate a large intake of resin. Such prescriptions may prove burdensome and expensive. Furthermore, in most instances, reduction in the dietary sodium is necessary if even these large quantities of exchanger are to be useful in influencing the retention of sodium. Hence, studies have been undertaken to determine the efficiency of a chelating agent such as ethylene diamine tetraacetic acid (EDTAA) alone or in combination in altering the

TABLE 1

INFLUENCE OF CARBOXYLIC EXCHANGERS UPON FECAL EXCRETION OF UNIVALENT CATIONS, CHLORIDE, AND NITROGEN* DURING MAINTENANCE ON DIETS OF VARIABLE SODIUM CONTENT

Resin	Specimens	Fecal excretion per gram of resin				
		Na (meq) Mean ± S. D.	K (meq) Mean ± S. D.	Na and K (meq) Mean ± S. D.	Cl (meq) Mean ± S. D.	N (gms) Mean ± S. D.
RCOOH	26	0.57 ± 0.7	1.12 ± 0.9	1.69 ± 1.2	0.03 ± 0.04	0.03 ± 0.03
$RCOONH_4$	8	0.58 ± 0.4	0.72 ± 0.3	1.30 ± 0.6	0.04 ± 0.04	0.02 ± 0.02
RCOOK	6	0.35 ± 0.5	1.36 ± 0.7	1.72 ± 0.9	0.02 ± 0.01	0.04 ± 0.02
RCOO Ca RCOO	9	0.20 ± 0.1	0.50 ± 0.3*	0.65 ± 0.2*	0.03 ± 0.02	0.03 ± 0.01
RCOONa	3	1.54 ± 0.6*	0.89 ± 0.5	2.44 ± 1.2	0.02 ± 0.02	0.03 ± 0.02

* Significantly greater or less than the effects observed with RCOOH ("p" less than 0.05).

TABLE 2

CONTENT OF ELECTROLYTES AND NITROGEN IN FECES OF PATIENTS ON A VARIABLE SODIUM INTAKE AND RECEIVING CORTISONE OR ACTH TOGETHER WITH CARBOXYLIC RESINS

Resin	Specimens	Excretion per gram of resin				
		Na (meq) Mean ± S. D.	K (meq) Mean ± S. D.	Na and K (meq) Mean ± S. D.	Cl (meq) Mean ± S. D.	N (gms) Mean ± S. D.
RCOOH 50% and RCOOK 50%	14	0.26 ± 0.1	0.82 ± 0.4	1.05 ± 0.5	0.02 ± 0.02	0.03 ± 0.01
RCOOH 50% and RCOOK 50% and Cpd E, 0.2–0.3 g/d	8	0.28 ± 0.2	1.35 ± 0.3*	1.63 ± 0.5*	0.01 ± 0.01	0.02 ± 0.01
RCOOH 50% and RCOOK 50% and ACTH, 0.1 g/d	8	0.42 ± 0.2	1.34 ± 0.4*	1.76 ± 0.4*	0.02 ± 0.02	0.03 ± 0.02

* Significantly greater than changes observed with RCOOH (50%) and RCOOK (50%) mixtures administered without hormones.

fecal content of univalent ions. The results, presented in TABLE 3, indicate that in this small group of experiments EDTAA when given alone in small amounts, 4 grams per day, failed to raise the excretion of sodium or of potassium. Preliminary studies, in which 10:1 mixtures of hydrogen form carboxylic exchanger and EDTAA were ingested for a total of 44 grams per day, are available but have not been tabulated. There is no evidence that the combination raises the efficiency of the resin in augmenting the excretion of univalent positive charged ions.

TABLE 3

FECAL COMPONENTS WITH OR WITHOUT INGESTION OF CARBOXYLIC RESIN OR ETHYLENE DIAMINE TETRAACETIC ACID (EDTAA)

Therapy	Specimens	grams/day	Excretion per day*				
			Na (meq) Mean ± S. D.	K (meq) Mean ± S. D.	Na and K (meq) Mean ± S. D.	Cl (meq) Mean ± S. D.	N (gms) Mean ± S. D.
Controls	23	0	3.9 ± 4.5	9.7 ± 4.8	13.6 ± 7.9	1.1 ± 1.7	0.9 ± 0.4
RCOOH	26	33.8	20.9 ± 25.4†	30.6 ± 22.2†	51.4 ± 45.1†	0.8 ± 0.6	0.9 ± 0.5
EDTAA	3	4	1.7 ± 2.0	6.4 ± 2.5	8.0 ± 4.5	0.3 ± 0.6	0.8 ± 0.4

* Hence not comparable with data in TABLES 1 and 2.
† Significantly greater than controls.

TABLE 4

FECAL CONSTITUENTS DURING INGESTION OF RESIN MIXTURES AND DIETS OF VARIABLE SODIUM CONTENT*

Mixture	Specimens	Excretion per gram of resins				
		Na (meq) Mean ± S. D.	K(meq) Mean ± S. D.	Na and K (meq) Mean ± S. D.	Cl (meq) Mean ± S. D.	N (gms) Mean ± S. D.
RCOOH	26	0.57 ± 0.7	1.12 ± 0.9	1.69 ± 1.2	0.03 ± 0.04	0.03 ± 0.03
RCOOH (50%) RSO_3H (50%)	6	0.39 ± 0.4	0.46 ± 0.3	0.84 ± 0.5	0.01 ± 0.01	0.02 ± 0.01
RCOOH (50%) APE (50%)	5	0.23 ± 0.4†	0.47 ± 0.4†	0.74 ± 0.8	0.03 ± 0.02	0.03 ± 0.03
RCOOH (40, 59%) RCOOK (10, 29%) APE (50, 12%)	6	0.40 ± 0.2	0.68 ± 0.3	1.08 ± 0.3	0.02 ± 0.01	0.04 ± 0.02

* APE refers to alkylene polyamine exchanger. None of the data obtained with any of these resin combinations are statistically significantly different from those observed with RCOOH: ("p" values ranged from 0.09 to 1.00).

† When the excretion is expressed in relation to the intake of the cation exchanger the Na increases to 0.41 ± 0.6 and the K to 0.90 ± 0.7 milliequivalents per gram of resin. This rise is not statistically significant.

TABLE 4, given above, indicates that acidifying sulfonic and carboxylic exchangers in combination are no more efficient per gram of ingested resins than the latter agent alone in influencing the electrolyte content of feces. The same negative results were obtained in our studies with admixtures of alkylene polyamine exchangers in varying proportions. We realize that evidence from other laboratories has indicated a slight increase in efficiency, even when expressed in relationship to the total intake of resins.[3, 19] This was not present

TABLE 5

SERUM CHANGES DURING THE SHORT TERM INGESTION, 14 DAYS OR LESS, OF VARIOUS TYPES AND FORMS OF SYNTHETIC RESINS WITHOUT REGARD TO DIETARY CONTENT OF SODIUM

Resin	No. of periods	Daily Dosage	Changes in Serum					
			HCO_3 (meq/L) Mean ± S. D.	Cl (meq/L) Mean ± S. D.	Na (meq/L) Mean ± S. D.	K (meq/L) Mean ± S. D.	Ca (mgm %) Mean ± S. D.	P (mgm %) Mean ± S. D.
None	42	0	+0.5 ± 1.0	+0.1 ± 2.5	+0.5 ± 4.2	+0.1 ± 0.5	0.0 ± 0.4	0.0 ± 0.5
RCOOH	21	30.8	−4.8 ± 3.3*	+3.6 ± 4.8*	+0.9 ± 6.5	−0.7 ± 0.7*	−0.3 ± 0.7	−0.2 ± 0.8
$RCOONH_4$	9	38.3	−5.3 ± 3.9*	+4.4 ± 3.8*	+2.7 ± 5.5	−0.3 ± 0.6*	−0.1 ± 0.5	−0.4 ± 0.5
RCOONa	3	40.0	−0.5 ± 2.3	−0.1 ± 2.3	+2.3 ± 2.3	+0.2 ± 0.7	—	−0.2 ± 0.4
RCOOK	5	49.0	+1.4 ± 1.1	+1.8 ± 3.1	+0.6 ± 6.2	+0.5 ± 1.2	−0.2 ± 0.5	+0.7 ± 0.3*
RCOO \\ Ca / RCOO	10	42.3	+0.3 ± 2.4	−2.1 ± 0.9*	−0.6 ± 4.5	−0.2 ± 0.4	−0.4 ± 0.7	0.0 ± 0.5
RCOOH (50%) and RSO_3H (50%)	5	36.0	−5.7 ± 4.3*	+8.3 ± 5.0*	−7.4 ± 7.9*	−0.8 ± 0.5*	+0.1 ± 1.7	+0.2 ± 1.2
RCOOH (50%) and APE (50%)	5	80.0	−2.7 ± 2.7*	+0.4 ± 7.0	−2.8 ± 5.3	−0.5 ± 0.4*	−0.5 ± 0.2	−0.2 ± 0.2
RCOOH (40, 59%), RCOOK (10, 29%) and APE (50, 12%)	7	60.0	+1.0 ± 2.7	+2.9 ± 2.4*	+2.1 ± 5.3	+0.1 ± 1.0	−0.3 ± 0.3	−0.4 ± 0.4

* Significantly different from controls as indicated by "p" values.

in our studies nor is there any evidence that the efficiency of the cation exchangers themselves was raised. It is to be noted that fecal excretion of chloride and nitrogen was of the same order of magnitude irrespective of the exchangers used.

TABLE 5 contains the changes in serum constituents at the end of short terms of therapy, 14 days or less, with these various resin combinations. It is readily evident that the hydrogen and ammonium forms of the carboxylic exchanger produce comparable degrees of acidosis, hyperchloremia and, at the same time, lower the serum potassium concentrations. Non-acidifying forms of this resin such as sodium, potassium, and calcium do not produce these changes when administered in approximately the same amounts. The alterations in serum phosphate and in serum chloride during potassium, and during calcium form carboxylic resin therapy, are statistically significant but unexplained.

The studies of serum components during the ingestion of combinations of acidifying carboxylic and sulfonic resins, presented in TABLE 5, again reveal hyperchloremia, acidosis, and lowered serum potassium levels. In addition, a statistically significant decrease in serum sodium occurred. From TABLE 5 it is also apparent that a mixture of hydrogen form carboxylic resin and of an alkylene polyamine exchanger in equal parts did not result in hyperchloremia, but decreases in both the serum CO_2 content and the serum potassium concentration did appear. Addition of small amounts of the potassium form resin and reduction in the relative proportion of the alkylene polyamine exchanger resulted in a resin mixture which produced only minor changes, if any, in the serum constituents under study.

Comment

Keeping in mind the limitations imposed by the miscellaneous character of the subjects and the variable sodium content of the diets, certain conclusions can be drawn from the data which have been presented:

(1) ACTH or cortisone therapy did not interfere with resin-induced increases in fecal sodium but may have augmented the excretion of potassium.

(2) No enhancement of fecal electrolyte excretion has been observed with ethylene diamine tetraacetic acid.

(3) The possibility that the ability of the sulfonic resins to bind cations at low pH values, such as those present in gastric juice, would increase the stool output of sodium has not been realized.

(4) The combination of anion-cation exchangers did not increase the per gram excretion of sodium and potassium either in terms of the total resin dosage or of the cation resin alone.

(5) Anion-cation resin mixtures minimized acidosis and might therefore be useful in those instances of renal disease in which an undesirable degree of acidosis may be expected to supervene.

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THE ROLE OF THE GASTRO-INTESTINAL TRACT IN THE ADAPTATION OF THE BODY TO THE PREVENTION OF SODIUM DEPLETION BY CATION EXCHANGE RESINS

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The paucity of sodium appearing in the fecal contents has intrigued investigators for many years. Until the advent of the flame photometer its quantitative estimation has been a difficult problem. With the demonstration by Dock[1] of the ability of cation exchange resins to increase the fecal excretion of sodium, a new tool has become available for the study of electrolyte absorption by the intestinal tract.

Hitherto, most of our exact knowledge of the behavior of the gut toward water and electrolytes stems from the classic studies of Visscher[2, 3] using labelled isotopes to follow the passage of these substances across the intestinal membrane. Briefly summarized, these investigations have revealed that sodium and water are passing simultaneously in both directions regardless of their relative concentrations in the blood and gut. Quantitative measurements have revealed that an amount equal to the entire sodium content of the dog's blood plasma may be transferred from blood to gut and back again every ninety minutes. This would amount in a sixty kilogram man to a turnover rate of sodium in the intestinal tract as high as 162 grams daily. Visscher showed, moreover, that the net absorption of sodium and water, that is, the amount leaving, minus the amount entering the gut, was least in the upper intestinal tract and greatest in the colon where it approached a unidirectional flow from gut to blood. The passage of the sodium ion from the intestinal lumen to the blood may take place against a high concentration gradient and cannot be explained by the laws of osmosis and diffusion. An active energy consuming process must be invoked. Clark[4] working in Visscher's laboratory was the first to point out a relationship between the adrenal glands and the rate of transfer of sodium chloride across the intestinal membrane.

During the course of our studies of the clinical use of cation exchange resins in the treatment of edema we have been impressed by two findings: first, the variability from patient to patient and in the same patient at different times of the amount of sodium removed in the feces by a given dose of resin; and second, the absence of any evidence of depletion of sodium from the body even after prolonged resin administration.

TABLE 1 demonstrates the wide range of fecal sodium excretion induced per each gram of resin ingested by a series of patients with edema due to various causes. These patients were fed either a sulfonic resin in the hydrogen cycle (Permutit Z)** or a carboxylic resin (Amberlite XE96)‡ in mixtures of different proportions of the ammonium and potassium cycles. There was no consistent correlation between the amount of sodium removed by the resins and the cycle in which they were fed. It will be noted that in one patient alone (M. L.) the amount of sodium removed per gram of resin varies from 0.25 to 0.80 m.eq. and similar widely scattered values are found for the other patients. In a larger series of patients even greater variations have been observed and this has been a uniform observation of other investigators in this field. Attention is called to the fact that in all but two instances, shown in TABLE I, fecal potassium excretion far exceeded that of sodium.

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TABLE 1

CHANGE IN FECAL EXCRETION OF CATIONS INDUCED BY ORAL ADMINISTRATION OF CATION-EXCHANGE RESINS*

Patient	Resin	Dose gm./day	Number of days	Sodium		Potassium	
				Total m.eq./day	m.eq./Gm. resin	total m.eq./day	m.eq./Gm. resin
M. L.	Permutit A	40	3	+16.0	+0.25	+24.0	+0.60
		60	9	+22.0	+0.37	+12.1	+0.21
	Amberlite (1)†	45	9	+36.1	+0.80	+83.3	+1.85
	Amberlite (2)	60	7	+33.0	+0.55	+72.0	+1.20
	Amberlite (2)	80	9	+27.7	+0.35	+96.0	+1.20
F. C.	Amberlite (3)	25	9	+7.9	+0.32	+54.0	+2.16
	Amberlite (3)	50	6	+15.0	+0.30	+78.0	+1.56
H. A.	Amberlite (1)	45	9	+33.9	+0.75	+12.0	+0.27
G. D.	Amberlite (2)	40	6	+20.8	+0.52	+92.8	+2.32
C. T.	Amberlite (4)	60	6	+15.6	+0.26	+73.1	+1.22

* The values represent the increase (+) in cation excretion during resin therapy as compared with excretion during a prior control period with approximately the same dietary intake.

† Amberlite (1) was composed of approximately two-thirds of the ammonium form and one-third of the potassium form (potassium equaled 5.2%). This was Resodec. Amberlite (2) contained equal parts of ammonium and potassium forms (potassium equaled 9.4%). Amberlite (3) was the potassium form (potassium equaled 18.7%). Amberlite (4) was the ammonium form.

The absence of signs of sodium depletion, even when large doses of resin are given, is exemplified in FIGURE 1. In this patient a sulfonic acid resin was administered in increasing doses up to 100 grams daily until acute symptoms of weakness, nausea, and muscular cramps appeared, whereupon the resin was stopped. It will be noted that there was a sharp drop below the average normal in the serum levels of calcium and potassium with a resultant hypokalemic alkalosis indicated by a marked rise in serum CO_2 combining power. An electrocardiographic tracing at this time revealed low T waves and prolonged QT interval thus substantiating the chemical signs of potassium depletion. *The serum level of sodium, however, remained remarkably constant and well within normal limits during this period of body depletion of other cations.*

TABLE 2 demonstrated a balance study done of this patient following a period of resin withdrawal. It will be noted that when resin administration

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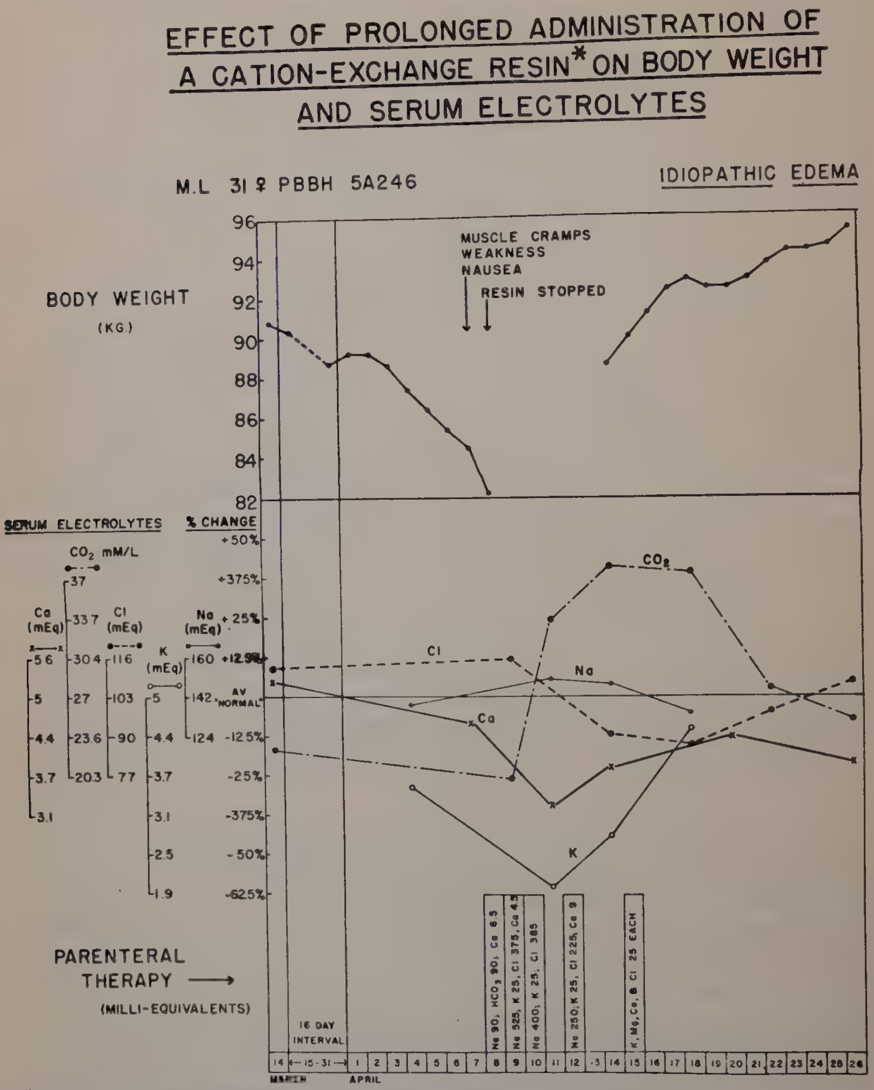
** Permutit Z was kindly supplied by the Permutit Company of America.

‡ Amberlite XE96 was supplied through the generosity of the Smith, Kline & French Co.

was resumed, this time a carboxylic exchanger, there was an immediate outpouring of sodium in the stool with a resultant negative sodium balance. As



EFFECT OF PROLONGED ADMINISTRATION OF A CATION-EXCHANGE RESIN* ON BODY WEIGHT AND SERUM ELECTROLYTES

* PERMUTIT-Z RESIN ADMINISTERED IN FOLLOWING DOSAGE: 60 GRAMS DAILY 2/18-2/28; NO RESIN 3/1-3/4; 60 GRAMS DAILY 3/5-3/16; 75 GRAMS DAILY 3/17-3/29; 100 GRAMS DAILY 3/30-4/7.

FIGURE 1.

resin administration was continued, however, stool sodium excretion decreased steadily until after six days a positive sodium balance was attained. A similar progressive decline in stool calcium excretion occurred during continued resin administration but no such change occurred in the excretion of potassium.

Indeed, there appeared to be some tendency for potassium excretion to increase as sodium excretion fell. This suggests that some homeostatic mechanism comes into play to conserve body sodium at the expense of potassium.

After eighteen months of continuous resin therapy, further studies of water and electrolyte distribution were carried out in this patient through the kindness of Doctor Francis Moore.[5] Measurements were made of total body water with deuterium oxide and total body sodium[6] and potassium[7] by isotope dilution. Extracellular fluid was measured by the thiosulfate method.[8]* Total body sodium was found to be 55.3 and potassium 22.2 m.eq./kilogram body weight. The patient was quite obese and normal values for her age and body weight, according to data obtained in Doctor Moore's laboratory, would be in the neighborhood of 32 m.eq. of sodium and 30 m.eq. of potassium per kilogram body weight. Admittedly, there is a wide range of normal for both these values but even after correcting for edema fluid the total body sodium remains above and potassium below the limits of the normal range. At least, it can be said, therefore, that from these studies there is no evidence of sodium depletion. Rather, the findings suggest that there is an accumulation of sodium in the body at the expense of potassium.

TABLE 2

A COMPLETE BALANCE STUDY SHOWING EFFECT OF A MIXTURE OF THE AMMONIUM AND POTASSIUM FORMS OF AMBERLITE XE96 RESIN (RESODEC) ON ELECTROLYTE EXCRETION

M. L., F. Age 32, Idiopathic Edema

Period	Resin	Sodium			Potassium			Calcium			Magnesium		
		Stool	Urine	Balance	Stool	Urine	Balance	Stool	Urine	Balance	Stool	Urine	Balance
3 days	*gm./ day*	*m.eq./ day*	*m.eq./ day*	*m.eq./ day*	*m.eq./ day*	*m.eq./ day*	*m.eq./ day*	*m.eq./ day*	*m.eq./ day*	*m.eq./ day*	*m.eq./ day*	*m.eq./ day*	*m.eq./ day*
I	0	1.0	6.0	+27.8	12	73	+37	44.1	5.9	+15.0	10.7	8.8	+12.7
II	45	71.0	2.9	−39.1	73	77	−40	61.0	5.1	−1.1	13.6	5.1	+13.5
III	45	30.0	8.7	−3.9	46	55	+19	23.5	7.4	+33.7	9.1	3.5	+19.6
IV	45	10.4	0.8	+23.6	167	34	−91	17.1	6.1	+41.8	23.2	1.2	+0.8

Daily intake of sodium 34.8 m.eq., potassium 110 m.eq., calcium 65 m.eq., magnesium 32.2 m.eq.

Mateer and his coworkers at Yale[9] have demonstrated that when resins are administered to patients with nephrosis while they are ingesting a diet containing negligible quantities of salt there occurs no increase in fecal sodium. When dietary sodium is increased in these same patients, however, resin administration results in a rise in stool sodium. To a limited extent, therefore, the amount of sodium extracted from the intestinal contents by the resin varies directly with the amount of sodium ingested. This correlation is far from close, however, and it is apparent from the above observations that some mechanism exists in the body for the conservation of body sodium by increased net absorption from the gut.

Berger and Steele[10] have shown that the action of resins in removing sodium from the intestinal tract is less pronounced in patients who are accumulating edema and who show very low sodium excretion in the urine than in those patients who are nonedematous or who have a normal urinary excretion of sodium. Here it would appear that the mechanism which operates in edematous states to reduce the urinary excretion of sodium acts in a similar manner on the intestinal membrane.

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* We are indebted to Doctors Anthony James, Isidore Edelman, and Richard Cardozo for the determinations of total body water, sodium and potassium and thiosulfate space.

An explanation of this mechanism was suggested by Berger[11] when they demonstrated that the administration of large doses of desoxycorticosterone significantly reduced the amount of sodium removed by cation exchange resin from the intestinal tracts of rats and of man. We have approached this problem from the opposite direction by studying the effect of the administration of cation exchange resins to human subjects deprived of their adrenals. The first opportunity which presented itself was in the treatment of a patient suffering from hypertension and glomerulonephritis who had been subjected to bilaterial adrenalectomy in an attempt to delay the progression of his disease. In spite of this measure, he still exhibited marked generalized edema. In an attempt to control this, continuous administration of a cation exchange resin was carried out. The dramatic results are shown in TABLE 3. When this patient was given 45 grams of resin daily for three days he excreted the enormous quantity of 252 m.eq. of sodium each day in his stools or an excess of 224 m.eq. over and above the control level. This amounts to 13.1 grams of sodium chloride daily or 5.0 m.eq. per gram of resin, a figure five fold as great as any we have found in individuals with normal adrenal function and approximating the maximum combining capacity of the resin. Needless to say, his edema promptly disappeared. Fortunately resin was discontinued before an Addisonian crisis was precipitated.

TABLE 3

EFFECT OF RESIN ADMINISTRATION IN A PATIENT WITH CHRONIC GLOMERULONEPHRITIS BILATERAL ADRENALECTOMY

K. M.

Period 3 days	Resin gm./d.	Na	K	Ca	P
		m.eq./d.		mg./d.	
I	0	28.0	28.5	712	534
II	45	252.5	64.0	663	327
III	0	65.0	44.0	556	334

Maximum sodium excretion = 5.0 m.eq./gm. resin or 13.1 gms. NaCl per day in excess of the control value.

In order to determine the effect of exogenous desoxycorticosterone on the fecal loss of sodium induced by resin in patients who were unable to adjust their own adrenal salt-retaining hormone, balance studies were carried out in two patients with well documented Addison's disease. The patients were maintained on a constant diet and salt intake except that additional salt had to be given after the initial period of resin administration in the first study, because of the development of acute symptoms of sodium depletion. The results of the first study are shown in FIGURE 2. The study was divided into three-day periods. Prior to its commencement the patient was receiving treat-

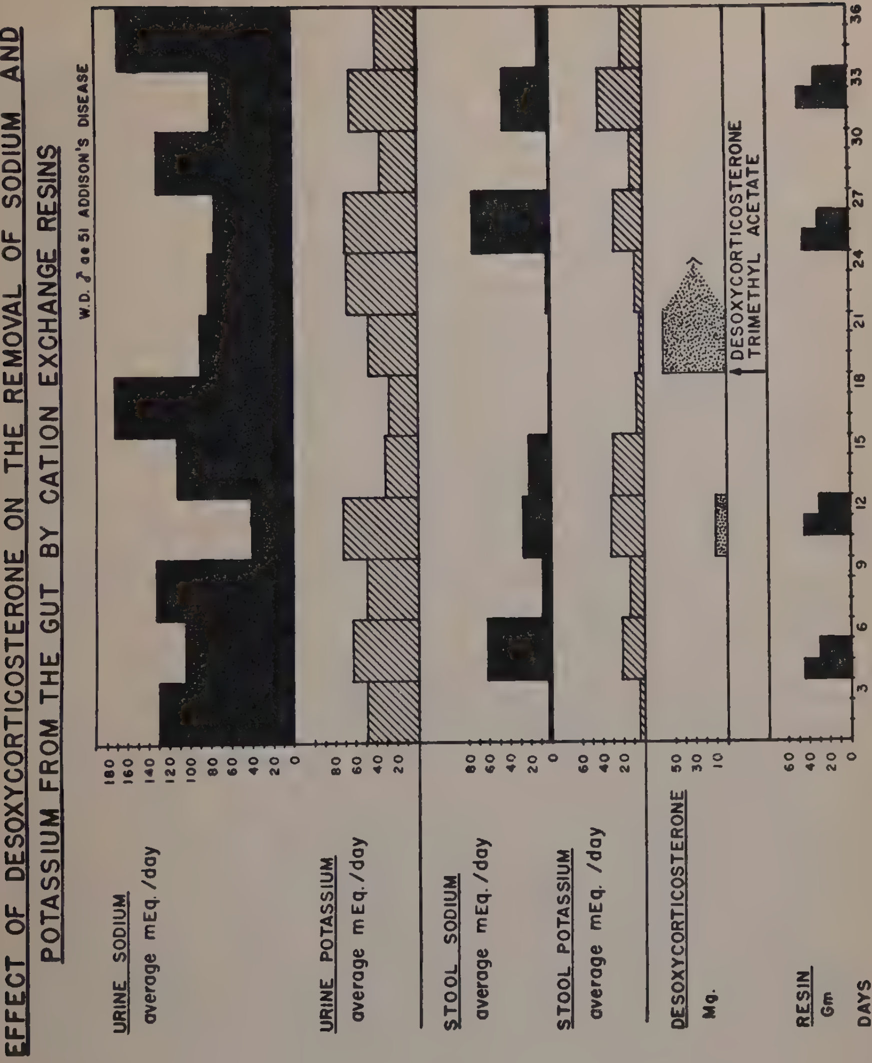
EFFECT OF DESOXYCORTICOSTERONE ON THE REMOVAL OF SODIUM AND POTASSIUM FROM THE GUT BY CATION EXCHANGE RESINS
W.D. ♂ ae 51 ADDISON'S DISEASE
URINE SODIUM
average mEq./day
180
160
140
120
100
80
60
40
20
0
URINE POTASSIUM
average mEq./day
80
60
40
20
STOOL SODIUM
average mEq./day
80
60
40
20
0
STOOL POTASSIUM
average mEq. /day
60
40
20
DESOXYCORTICOSTERONE
Mg.
50
30
10
DESOXYCORTICOSTERONE
TRIMETHYL ACETATE
RESIN
Gm
60
40
20
0
DAYS
3
6
9
12
15
18
21
24
27
30
33
36

FIGURE 2.

ment with cortisone 12.5 mgs. twice daily by mouth. This treatment was continued throughout the study. After a three-day control period in which the fecal sodium averaged 3 m.eq. daily, resin was administered in a dose of 45 grams the first day, 30 grams the second and none the third. The fecal excretion of sodium promptly rose to 62 m.eq. per day, whereas fecal potassium rose only from 6 to 21 m.eq. per day. There was very little compensatory fall in urinary sodium excretion. Following another control period, during which the daily fecal excretion of sodium and potassium fell to 9.0 and 14.0 m.eq. respectively, the same dose of resin was administered simultaneously with a daily injection of 10 mgs. of desoxycorticosterone in oil. During this period, the fecal excretion of sodium rose only to 27 m.eq. and there was a slightly greater increase in fecal potassium to 31.7. There was a sharp fall in urinary sodium output to 40 m.eq. daily. Subsequent study was made of the effect of an intramuscular injection of a suspension of the trimethyl acetate of desoxycorticosterone which, in the dose of 60 mgs., has been shown to maintain adequate salt retention in patients with Addison's disease for approximately one month. Six days after one injection of 60 mgs. of this material, administration of resin was resumed in the same dose as during the previous studies. There resulted an increase in fecal output of sodium to 76 m.eq./day, again markedly exceeding that of potassium which rose only to 27.7 m.eq. per day. It is obvious that this quantity of desoxycorticosterone was insufficient to prevent the excessive removal of sodium from the intestinal tract although it was sufficient to maintain a fairly normal urinary sodium output.

A final observation was made of the effect of a carboxylic resin combined with an anion exchange resin. The dose used contained the same quantity of cation exchange resin as in the previous experiments. Again, an excessive loss of sodium from the intestinal tract occurred although not quite as great as in the previous experimental periods in which the cation exchange resin was given alone. It is noteworthy that the effect of resin on the loss of potassium in the stool was again less than is seen in individuals with normal adrenal function and, in all instances of administration of resin without desoxycorticosterone, fecal sodium exceeded fecal potassium excretion in these experiments.

TABLE 4 shows a second study on this same patient in which his salt intake was maintained constant at 14.56 grams per day. Here again we see the excessive increase in fecal sodium output during resin administration amounting to approximately 2 m.eq. per gram of resin and the sharp reduction in this value when desoxycorticosterone was given. Although these values are not as high as those seen previously in the patient with edema and bilateral adrenalectomy, they are still far in excess of those observed in patients with functioning adrenals. Again, fecal sodium exceeds fecal potassium except when desoxycorticosterone is given. In the second patient with Addison's disease (TABLE 5) similar findings were obtained. During resin therapy alone this patient excreted more sodium than potassium in his stools. When desoxycorticosterone was added in doses of 20 mgs. daily this relationship was reversed, the fecal sodium excretion being reduced from 20.0 to 4.1 m.eq./day and fecal potassium rising from 18.3 to 35.0 m.eq./day.

This reciprocal behavior of fecal potassium appears to us to be highly significant. In subjects with intact adrenals cation exchange resins almost invariably induce a higher fecal excretion of potassium than of sodium. In this respect the studies of Peters *et al.*[12] are of interest. They noted that the administration of ACTH or cortisone in conjunction with cation exchange resins uniformly induced hypokalemia whereas the same amount of resin alone did not. Their data suggest, moreover, that these agents acting together resulted in a greater loss of potassium in the feces than did the resin alone. To this

TABLE 4

EFFECT OF RESINS ON ELECTROLYTE EXCRETION IN ADRENAL INSUFFICIENCY WITH AND WITHOUT DESOXYCORTICOSTERONE

W. D.

Date	Treatment*			Urine		Stool	
	1	2	3	Na	K	Na	K
				m.eq./d.		m.eq./d.	
5-14	12.5			203	49.7	14.2	2.0
5-15	12.5						
5-16	12.5						
5-17	12.5	30		159	67.7	72.5	28.3
5-18	12.5	30					
5-19	12.5	30					
5-20	12.5	30	20	82	71.3	42.5	52.5
5-21	12.5	30	20				
5-22	12.5	30	20				
5-23	12.5	30		148	57.3	70.8	44.2
5-24	12.5	30					
5-25	12.5	30					

* 1. Cortisone, mgs./d.
2. Resodec, gms./d.
3. DOCA, mgs./d.

TABLE 5

EFFECT OF RESINS ON ELECTROLYTE EXCRETION IN ADRENAL INSUFFICIENCY WITH AND WITHOUT DESOXYCORTICOSTERONE

G.P. Date	Treatment*			T.V. cc./d.	Urine					Stool				Serum	
	1	2	3		Na	K	Ca	Cl	PO$_4$ mM/d.	Na	K	Ca	PO$_4$ mM/d.	Na	K
					m.eq./d.					m.eq./d.				m.eq./l.	
4-21	12.5	20		973	134	61.3	2.78	165.0	22.7	20.0	18.3	21.8	4.8	133	4.0
4-22	12.5	15													
4-23	12.5	20													
4-24	12.5	20	20	835	37	67.5	2.48	67.6	27.2	4.1	35.0	27.4	5.3	145	5.2
4-25	12.5	20	20												
4-26	12.5	20	20												

* 1. Cortisone, mgs./d.
2. Resodec, gms./d.
3. DOCA, mgs./d.

extent, therefore, the induction of a state of hyperadrenalism brought about a response to resins, namely an excessive removal of potassium from the body, that was the opposite of what we have observed in patients with adrenal insufficiency where the loss of sodium exceeds that of potassium.

Conclusions

From Visscher's work[2, 3] it is apparent that enormous quantities of sodium may be present in the intestinal tract and potentially capable of combining with cation exchange resins; and yet in a series of ten patients treated with resins of the carboxylic type for periods ranging from six months to more than two years we have yet to encounter a single instance of proven sodium depletion.

The evidence presented above would suggest that this is due, at least in part, to a compensatory increase in the elaboration of the salt retaining hormone by the adrenal cortex and that this hormone acts on the intestinal mucous membrane in a manner similar to its action on the renal tubule and the sweat and salivary glands. Under its influence the net absorption of sodium from the gut is increased and the amount available for exchange with the resin correspondingly diminished. More valences on the resin then become available for exchanging with potassium and the amount of this ion attached to the resin increases as the amount of sodium diminishes. Our data do not indicate whether this is purely the result of mass action determined by the relative concentrations of ions in the gut, or whether an actual net increase of potassium excretion into the gut may be induced by the adrenal cortical hormone.

Conversely, when adrenal cortical activity is diminished or absent, the active absorption of sodium from the gut is lessened and a larger amount is available for combination with resins. Fewer valences are left open for potassium and its removal in the feces will be correspondingly diminished. Under these circumstances, the administration of desoxycorticosterone in adequate amounts can restore the normal relationship of sodium and potassium concentration in the gut and therewith the normal excess of potassium over sodium removed by resins.

Finally, the question will be raised, why does the individual with adrenal insufficiency not continually lose sodium by the enteric route? A final answer cannot be given at this time but it is noteworthy that relatively large amounts of salt-retaining hormone, in the range of 10 to 20 mgs. daily, are necessary to reduce the excessive fecal loss of sodium induced by resins in the adrenal insufficient patient. Such doses would soon reduce sodium in sweat and urine to the vanishing point. This would imply, but certainly not prove, that the intestinal mucous membrane is less responsive to desoxycorticosterone than other end organs and that, under normal conditions, the adrenal hormones are not essential to the reabsorptive function of the gut. The hypothesis suggests itself that there may be a facultative and an obligatory reabsorption of salt and water from the gut, analogous to the mechanisms observed in the kidney. The facultative reabsorption might be considered that portion under hormonal control whereas the obligatory reabsorption is regulated primarily by the autogenous activity of the intestinal mucosa itself. It would appear that under normal conditions of intestinal activity and salt concentrations, the obligatory reabsorptive process is sufficient to prevent the loss of sodium in the feces. Only under the stress of sodium depletion is the faculative reabsorptive process called into play.

Summary

In the treatment of edema with cation exchange resins a marked variability from patient to patient in the amount of sodium removed in the feces has been noted and no evidence of any depletion of body sodium has ever been obtained even after prolonged resin administration. There appears to be a tendency for the amount of fecal sodium to diminish and potassium to increase as resin administration is continued. In one patient, treated for two years, total body sodium was increased and potassium diminished as compared to normal values. Almost invariably the amount of potassium removed from the gut by resins was greater than that of sodium.

In patients with adrenal insufficiency the administration of resins induced a fecal loss of sodium several times as great as in subjects with normal adrenals, exceeding in all instances the fecal loss of potassium. The concurrent administration of desoxycorticosterone in relatively large doses reduced the amount of fecal sodium removed by resin in these patients to values in the same range as normal individuals and at the same time increased fecal potassium loss so that it exceeded that of sodium. It is postulated that the hormones of the adrenal cortex are capable of regulating the net absorption of sodium and potassium from the gut in a manner similar to their regulation of the excretion of these electrolytes in the urine, sweat, and saliva. This mechanism appears to operate only under the stress of sodium deprivation. Under normal circumstances, however, the autogenous activity of the intestinal mucosa is sufficient to reabsorb the major portion of the sodium in the large bowel.

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ELECTROLYTE AND WATER MOVEMENT ACROSS THE INTESTINAL WALL

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The nature and the rates of the transport processes occurring across the intestinal wall are the essence of the problems of intestinal absorption and excretion. In order to predict the usefulness of orally administered ion exchange resins in medicine it is essential that one know about the capacity of the intestinal mucosa at the several levels of the bowel to allow or to force the transport of ions in either direction across it. Across any biological membrane or barrier there may conceivably be simple diffusion of ions or molecules, with or without net transport, depending simply upon permeability and solubility phenomena. There may also be movement under electrical forces, either simple iontophoresis or electro-osmosis. And there may be other unknown types of transport or pumping mechanisms, such as carriers with circular or shuttling metabolic paths.

By virtue of the characteristics of the intestinal wall it is known that the concentrations of various ions can be very different in the intestinal lumen phase than in the blood plasma phase of the system in question. Concentration ratios for sodium, chloride, and hydrogen ions may be of the order of a hundred or more.[1] It is necessary to know how such concentration differences are produced in order to be able to predict the effects of agents which may immobilize or restrain various ions.

The Flux Rates for Sodium Between Intestinal Lumen and Blood

Experiments[2, 3] on dogs, using surgical prepared chronic Thiery-Vella loops of intestine, have shown that radio-sodium moves across the blood vessel-intestinal lumen barrier at higher rates under comparable concentration conditions at the oral than at the aboral end of the bowel. FIGURE 1 shows some of the data from representative experiments.

In these studies the radio-sodium was placed sometimes in the gut lumen and sometimes in the blood. In the latter case, the injection was made long enough before the studies were performed so that the blood and the various other fluids and tissues of the body had approached equilibrium with respect to isotope distribution. Studies were made of isotope movement in both directions by actual measurement because it is conceivable that the existence of a barrier (membrane) of finite volume, of variable composition and unknown degree of stirring might result in different measured quantities of isotope leaving and entering the gut lumen phase.

Barring complications resulting from a membrane of finite volume and variable composition it is axiomatic that $R_{\text{out}} - R_{\text{in}} = R_{\text{net}}$. Where R_{out} is the flux rate of a substance out of the gut lumen, R_{in} the comparable rate into, and R_{net} the rate of net transport.

Measurements have shown that the above equation is valid for the real case of the intestinal lumen-blood system, within the limits of accuracy of measurement, indicating that the complications listed above do not give rise to large factors of error. To make such estimations R_{out} and R_{net} have been measured directly in one set of experiments and R_{in} and R_{net} in another set on the same loops of intestine. In the first case R_{out} was calculated and in the second R_{in} was. The measured and the calculated values were substantially the same, within random errors, as is seen in FIGURE 2.

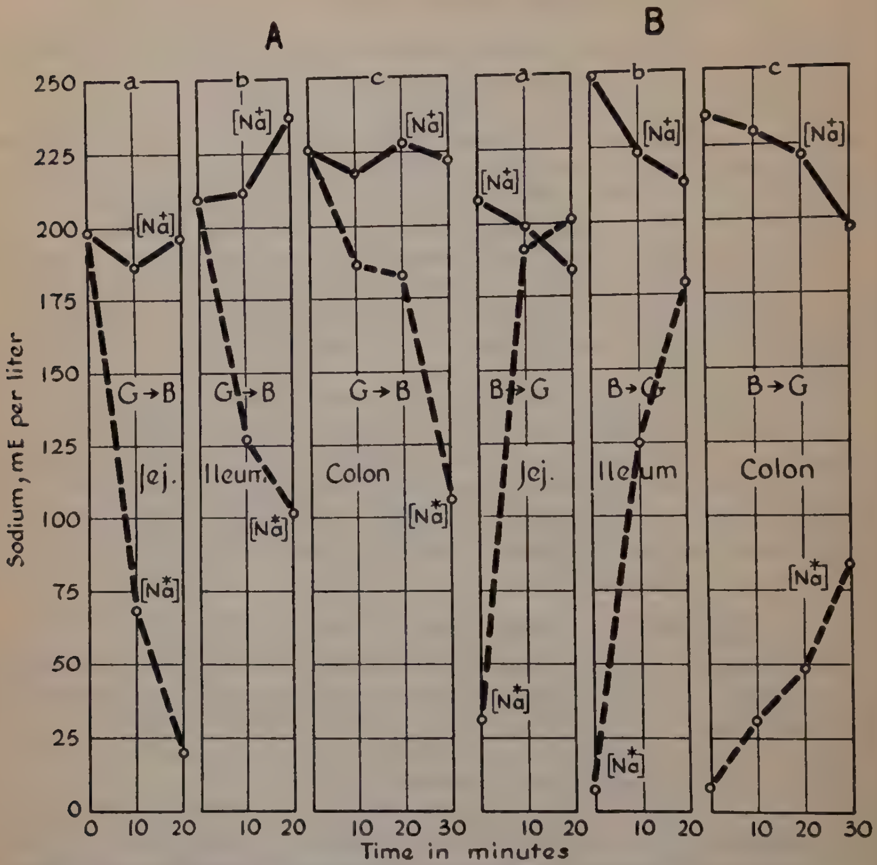

FIGURE 1. Changes in sodium concentration Na^+ and labeled sodium concentration Na^* in solutions placed in surgically prepared Thiery-Vella segments of intestine at various levels. In the parts of the graph marked A, a, b, & c, the isotope moved from gut to blood and in those marked B, a, b, & c, the isotope moved from blood to gut. The solutions placed in the intestine were originally isotonic mixtures of sodium chloride and sodium sulfate. The considerably lower rate of isotope equilibration at the aboral end of the intestine will be evident.

The most striking difference, aside from absolute flux rates, between oral and aboral intestinal membrane is the fact that the ratio between R_{in} and R_{out} is greater at the aboral end (see FIGURE 2). This ratio is obviously of importance to the efficiency of absorption. The larger the difference between the two fluids, if $R_{\text{out}} > R_{\text{in}}$, the more completely can a substance be cleared from

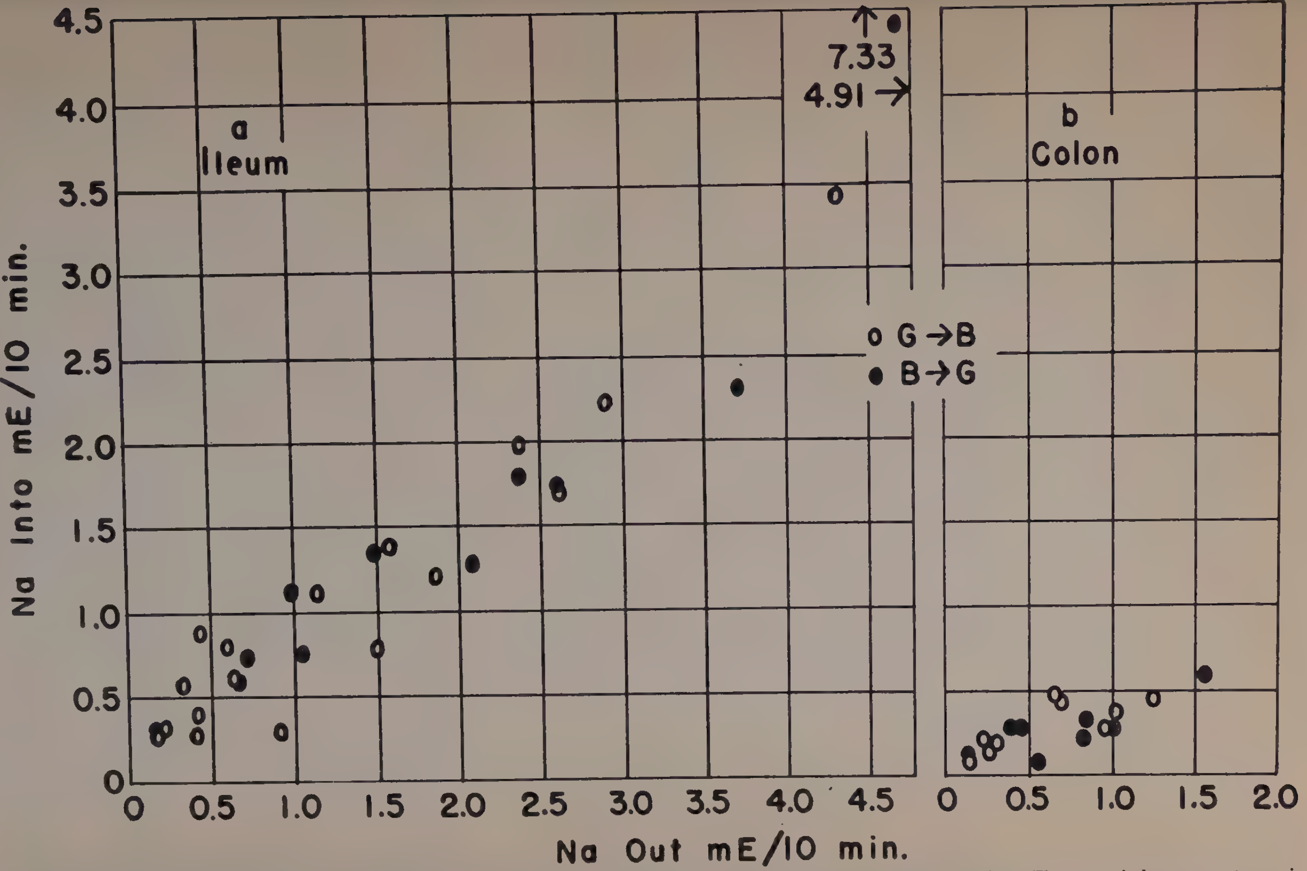

FIGURE. 2. The calculated flux rates for sodium movement into and out of intestinal segments are shown for ileum and colon. The open circles represent experiments in which the tracer was placed in the gut and the solid dots those in which the tracer was in the blood. The displacement of the points from the 45° line is the measure of net absorption which, it will be noted, is proportionately greater at particular absolute rates in the colon than in the ileum.

the intestinal lumen. The colon is an efficient sodium absorber, the ileum is less so and the upper jejunum and duodenum are very inefficient, under the conditions studied. Time is a factor in these processes and therefore must be considered in connection with the ambient [Na+] in the intestine at various levels with regard to the action of exchange resins. Intestinal hypo- or hypermotility will determine ionic concentrations, other factors being constant, and therefore the motor activity of the bowel should be considered in relation to the use of ion exchange substances.

The Dependence of Sodium Flux Upon Lumen Fluid Sodium Concentration

In experiments employing isosmotic mixtures of various proportions of sodium and magnesium salts it was found that the directional flux rates depended upon the [Na+] in the lumen. FIGURE 3 shows the results of a typical set of experiments. No tests were made with other univalent alkali metal ions, but it would not be impossible that the important factor would be the concentration in the gut fluid of all univalent cations, rather than of sodium ion alone. It would be worth while, it would seem, to test the latter hypothesis. The result would depend upon whether the directional flux rates for sodium and potassium were the same at comparable concentrations, at various levels of the gut. No studies have as yet been made to test these questions using tracer techniques. One old observation bears upon the question. It is well established that the concentration of potassium is higher than that of sodium in normal feces on ordinary diets. This end result would make it seem likely that, at least in the colon and rectum, sodium and potassium ions are sharply differentiated from one another in their treatment by the intestinal epithelium. The end result could occur either if $R^{\mathrm{Na+}}_{\mathrm{out}} \gg R^{\mathrm{K+}}_{\mathrm{out}}$ or $R^{\mathrm{K+}}_{\mathrm{in}} \gg R^{\mathrm{Na+}}_{\mathrm{in}}$.

The Absorption of Water and Ions Into Blood and Lymph

The lacteals in the villi could conceivably be channels through which a large share of intestinal absorption might occur. Lee and Grim[4] have cannulated the several small lymphatic channels visible in a segment of ileum and simultaneously cannulated the vein draining the same isolated segment. They have collected the lymph, the venous blood and samples of arterial blood before, during, and after the introduction of solutions with isotopes for water, sodium, and potassium into the lumen of the gut loop. Two unexpected findings were made. First, it was seen that mesenteric lymph tended to have isotope abundance ratios closer to arterial than to local venous blood plasma levels under most circumstances. Second, it was found that the local venous blood was not closer than ten per cent of equilibration with the gut contents. The first finding is tentatively interpreted as indicating that lymph from the lacteals is subsequently exposed at effective diffusion distances to large volumes of capillary blood which has not passed near the intestinal epithelium, and thus loses its excess of tracer material. The second finding is interpreted, again tentatively, to result from the fact that a relatively small fraction of the blood flowing through the wall of the intestine actually passes within effective diffusion distance of the epithelial surface. An alternative to this interpretation

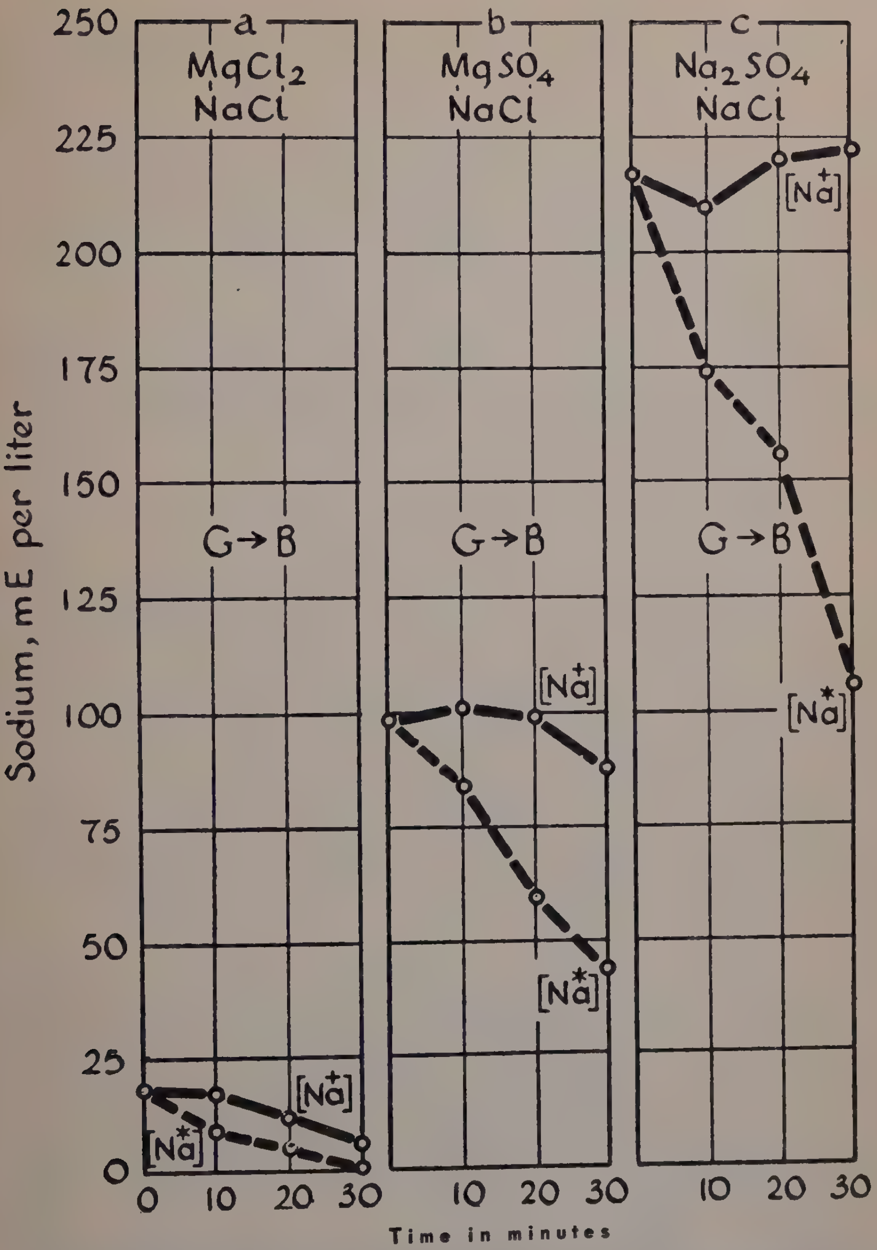

FIGURE 3. The rate of movement of isotopic sodium out of segments of ileum is seen to be dependent upon the concentration of sodium in the segment. Symbols as in FIGURE 1. The solutions employed were isotonic mixtures of the salts indicated at the tops of the graphs.

would be that the epithelial cell layer, which is really an interphase, rather than an interface or layer with negligible volume, is an extremely effective barrier against diffusion. It is not unlikely that both factors might be operative. Certainly the membrane interphase is not an inert diffusion barrier, because, if it were, net absorption would not occur from solutions of composition identical with plasma, which actually occurs. Furthermore, the differences in flux rates for various ions into and out of the lower gut as compared with the upper levels means that the barriers and/or active transport processes are morphologically determined. Thus, there is a basis for the supposition that the interphase is not as freely permeable to ions as is, for example, the capillary endothelium.

The above mentioned studies on lymph do not rule out the possibility that there may be a local transport of material via the lymphatics in the villi to blood vessels in the outer wall of the intestine. The observations do, however, prove that negligible amounts of water, sodium and potassium are transported through the large lymphatic channels.

The Influence of Adrenalectomy Upon the Net Flux of Ions and Water Across the Intestinal Membranes

Dennis and Wood[5] studied salt and water absorption in adrenalectomized dogs using techniques similar to those employed in other studies from this laboratory. They found that in the state of adrenal cortical insufficiency the net flux of sodium from ileal loop to blood was greatly depressed or reversed in direction. Potassium net flux was not as greatly depressed and was not observed to be reversed in sign under the conditions of their study. Net water movement was also diminished greatly by adrenal insufficiency. The changes were reversed by administration of adrenal cortex extract (Wilson). Since directional flux rates were not studied no conclusion could be reached as to the more intimate mechanism of the effect of adrenal insufficiency. It is obvious, however, that $R_{\text{in}}^{\text{Na}^+} \lesseqqgtr R_{\text{out}}^{\text{Na}^+}$ when $R_{\text{net}}^{\text{Na}^+} \lesseqqgtr 0$. Whether $R_{\text{in}}^{\text{Na}^+}$ increased or $R_{\text{out}}^{\text{Na}^+}$ decreased is as yet unknown.

Summary Discussion

Transport of material across the gut-blood interphase must be analyzed as a phenomenon in which net flux is the algebraic sum of directional fluxes. It is as yet impossible to indicate how large a fraction of the movement in either direction is due to simple diffusion, to iontophoresis, to electro-osmosis or to other mechanisms. The membrane phase, in part at least, determines the flux rates, which are not controlled simply by activities or concentrations.[2, 6] Since the directional rates are determining for net transport, the further analysis of factors controlling them is the next immediate step in studying the problem of intestinal absorption.

The functional activity of ion exchange resins in the bowel will depend in large part upon the action of the intestinal membrane in determining both the rates of movement of ions across the barrier and their concentration in the gut lumen phase. Moreover, the motility of the intestine at its various levels will also, of necessity, exert a controlling influence because the concentration of various ions and their rates of transinterphase flux differ at the several levels of the intestine.

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THE INTERPRETATION OF THE LABORATORY DATA IN THE CLINICAL USE OF THE CATION EXCHANGE RESINS

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The electrolyte alterations induced by the cation exchange resins depend primarily upon the specific cycle employed. An understanding of the characteristic electrolyte changes in plasma, urine, and stool produced by each cation cycle must underlie the judicious use of the proper cycle or combination of cycles in the therapy of specific electrolyte disturbances.

NH_4^+- and H^+-Cycle

The specific affinity of any cation exchange resin for other cations will be conditioned by the physical characteristics of the solution in which the exchanger is active.[1, 13] Accordingly, variations in sodium and potassium intake may directly influence the nature of any exchange.[2] In patients maintained on an appreciable salt intake, the NH_4^+- and H^+-cycle of the cation exchange resins will exert identical and characteristic effects on the electrolyte pattern of the plasma, urine and stool (FIGURE 1). In so far as the NH_4^+ or H^+ in these resins exchanges directly with potassium and sodium, the administration of these agents will induce a sizable increase in the stool content of sodium and potassium without simultaneous alterations in the fecal excretion of chloride or nitrogen.[3] As a direct consequence, the ingested sodium and potassium chloride is absorbed by the gastrointestinal tract, in part at least, as ammonium or hydrogen chloride. The plasma alterations will thereby simulate those produced by the administration of ammonium chloride or hydrochloric acid. The plasma chloride concentration will increase, the bicarbonate will fall, and a slight reduction in plasma, sodium, and potassium concentrations will ensue.[3, 4, 12, 13] If the metabolic acidosis is not compensated, variable reductions in plasma pH will also occur.

Total urinary output, the excretion of titratable acid, phosphate buffer, and ammonia all increase.[3] These changes comprise the usual compensatory, base-conserving response of the kidney to acidosis.[5] Coincidentally, sharp reductions in the urinary excretion of sodium and proportionately smaller reductions in the urinary excretion of potassium occur.[3, 4] Although it has been reported that acidosis evokes at least a transient increase in urinary sodium excretion,[5] this particular form of acidosis has not produced such changes.[3, 4] In part, this sustained reduction in urinary sodium and potassium excretion may result from the loss of these cations in the stool. It is of interest that the urinary volume increases despite the absence of a coexistent sodium or potassium diuresis.

The overall effects of NH_4^+ or H^+ cation therapy thereby include an exogenous loss of body potassium, presumably derived from intracellular sources, a less impressive negative water balance and little overall negative sodium balance.[3, 4, 6] This discrepancy between the effects on exogenous sodium and potassium balance reflects the relatively greater affinity of the resin for potassium and the more sluggish reduction in urinary potassium excretion. When normal amounts of sodium are ingested, the acid resins presently available hardly remove amounts of sodium adequate to produce a sustained negative external balance of this cation.

These effects of the NH_4^+ or H^+ cation exchange resins (acid resins) are considerably modified in those patients who are maintained on a salt-free diet.[2, 12] Under these circumstances, the amount of sodium available for exchange with the resin appears to be markedly reduced. Consequently, the administered cation is exchanged predominantly with potassium, augmenting the fecal excretion of this cation, with little increase in the sodium loss.[12] The plasma changes are far less impressive than those described above. A slight reduction in serum bicarbonate and potassium concentration has been noted without significant changes in the other plasma electrolytes.[2] This very mild acidosis may reflect the absorption of a few mEq of released NH_4^+- or H^+-ions with anions other than chloride. The changes in urine excretion are equally unimpressive and comprise only a slight reduction in potassium excretion. On a strict salt-free diet, this particular cycle of the cation exchange resin will produce only a negative exogenous potassium balance, with little effect on overall sodium balance.

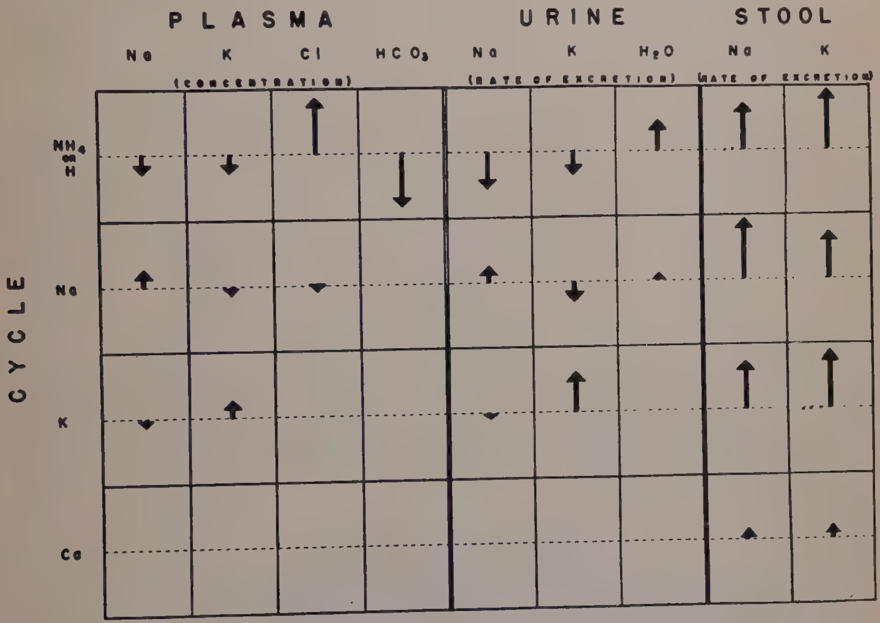

FIGURE 1. Electrolytes in plasma and excreta as a function of cycle of ion exchange resins.

K^+-Resin

The chemical alterations evoked by the K^+-cycle are far less conspicuous than those produced by comparable doses of the NH_4^+- or H^+-cycle (FIGURE 1).

In so far as the carboxylic resins display a greater affinity for potassium than for sodium, the administration of this particular cycle produces a marked increase in fecal potassium excretion and a proportionately smaller increment in the stool sodium content.[6] The absorbed potassium often induces a variable increase in the plasma potassium concentration, but no other consistent alterations in the plasma electrolyte pattern have been described. Despite prompt increases in urinary potassium excretion, this resin induces a positive exogenous potassium balance but little overall negative sodium balance.[3, 6]

Sodium Resin

In the gastrointestinal tract, this resin undergoes a predominant exchange of sodium for potassium. Accordingly, as with the potassium resin, prompt increases occur in the rate of fecal excretion of sodium and potassium[3, 16] (FIGURE 1). The absorbed sodium remains almost exclusively in the extracellular compartment, often inducing a slight increase in plasma sodium concentration. Otherwise, no consistent alterations in plasma electrolyte concentration occur. Although there is a secondary increase in the rate of urinary sodium excretion, the sodium balance tends to become positive. The fall in urinary potassium excretion, however, fails to inhibit a moderate loss of body potassium.

Calcium Resin

The affinity of these cation exchangers for bivalent cations exceeds that for univalent resins, and the Ca cycle, therefore, produces only a moderate increase in fecal sodium and potassium excretion. This cycle is less effective in withdrawing either sodium or potassium than the acid resin. Changes in plasma electrolyte concentrations and in overall electrolyte balance are unimpressive[6] (FIGURE 1).

Congestive Heart Failure

In the treatment of congestive heart failure, the primary goal usually consists in establishing a negative sodium and water balance. The prevention of any further salt and water retention is, therefore, imperative. While it has been demonstrated that the presently available cation exchange resins do not *per se* consistently induce a sizable sodium loss, the administration of the NH_4^+- or H^+-cycle in usual doses (50–100 grams per day) will efficiently inhibit the absorption of considerable quantities of sodium.[7, 13] In those patients maintained on a normal or slightly reduced salt intake, these agents will thereby prevent the daily absorption of 2–3 grams of sodium. Accordingly, such patients may be maintained on more palatable and nourishing diets. Over prolonged periods of treatment, however, the effectiveness of these resins in augmenting the fecal sodium loss appears to decrease.[8]

The development of a predictable hyperchloric acidosis will, in addition, initiate a mild water diuresis, if not a transient natriuresis. Three days of acid resin therapy administered in usual dosage to patients ingesting a considerable amount of salt (3–7 grams) will evoke an acidosis comparable in degree to that produced by the daily administration of 5–8 grams of ammonium chloride. Consequently, these resins may also serve to augment a mercurial diuresis. When used for this purpose, these exchange resins need only be employed intermittently for periods of three or four days.

Patients with underlying renal disease in whom the compensatory increase in the rate of urinary excretion of ammonia, buffer phosphate and titratable acid is relatively inadequate may develop an acidosis of such proportions as to produce overt clinical signs. Under these circumstances, the acid resins must be used with meticulous caution and their administration controlled with frequent plasma electrolyte determinations. Whether a prolonged acidosis, albeit compensated, will be well tolerated remains to be determined. In acute and chronic renal disease, where such an electrolyte disturbance may develop spontaneously, no characteristic irreversible damage attributable to the acidosis *per se* has been described. The affinity of these resins for the calcium contained in the gastrointestinal tract and the well known calcium-depleting effects of chronic acidosis suggest, however, that patients continued on long-term cation resin treatment should be maintained on small amounts of adjunctive calcium therapy.[9]

It should also be emphasized that the acid cycle resins evoke considerable losses of cellular potassium, sometimes replaced, in part at least, by extracellular sodium.[3, 4] It has been suggested that these effects may, in themselves, be harmful to a failing myocardium.[10] Furthermore, such a depletion of cellular potassium may often exaggerate the frequency and severity of the signs of digitalis toxicity.[11] Accordingly, most commercially available cation resins have been combined with the potassium cycle in the approximate ratio of 3 to 4 parts NH_4^+ or H^+ to 1 part K^+. Such a combination effectively inhibits any depletion of the potassium stores.[12] Additionally, however, it reduces to some slight extent the fecal loss of sodium and the degree of the accompanying acidosis. Because of the affinity of these resins for potassium, considerable intakes of potassium may further reduce the efficiency of these resins.[13] In those subjects in whom the resins are used intermittently to augment a mercurial diuresis, it seems reasonable, therefore, to replace the body potassium only after the resin therapy has been discontinued. For the most part, the ratio of 3 or 4 parts NH_4^+ to 1 part K^+ appears to compromise the maximal effectiveness of the cation resins with the minimal loss of body potassium. In individual subjects, however, this ratio should be altered to achieve maximal benefit.

Apart from the beneficial influence of these resins in delaying or inhibiting salt retention and in augmenting a mercurial diuresis, the status of the presently available cation exchange resins in the treatment of congestive heart failure does not warrant overzealous enthusiasm. These resins frequently produce distressing gastrointestinal complaints.[9, 13] *Per se* they do not evoke sizable sodium losses.[6] In patients maintained on a strict salt-free diet, their administration is of questionable value.[12] In this circumstance, their tendency to produce potassium depletion may be rendered even more conspicuous. Furthermore, any increase in adrenal activity may reduce the fecal excretion of sodium.[14] In so far as circulatory failure may be associated with adrenal hyperfunction, it is conceivable that such patients are relatively resistent to the withdrawal of sodium by these agents.

The principles considered in regard to the use of the cation exchange resins in circulatory failure may also provide a basis for their administration in patients with cirrhosis and the nephrotic syndrome. Furthermore, these agents may ultimately prove of some value in the treatment of essential hypertension.[15] Once salt depletion has been achieved, their administration may permit a moderate increase in salt intake without a comparable retention of sodium. In so far as renal function may be reduced in the nephrotics of hypertensives, considerable care must be exercised to avoid the development of severe uncompensated acidosis.

Acute Renal Insufficiency

In acute renal insufficiency, the increase in plasma potassium represents the primary electrolyte disturbance, the correction of which may be lifesaving. Both the acid and sodium cycles of the cationic resins may be utilized for this purpose. The former will, of course, exaggerate any acidosis present prior to the onset of resin therapy. A severe pre-existing acidosis in the absence of functioning kidneys may therefore contraindicate the use of this particular cycle. Furthermore, the frequency of spontaneous hyponatremia in these patients argues against the use of a resin which may further reduce the plasma sodium concentration. Accordingly, in subjects in whom the acidosis and hyponatremia are particularly prominent, the sodium cycle may be the preferable choice. If fluid intake is sharply reduced, the exchange of resin sodium for the body potassium will reduce the extent of the hyperkalemia and coincidentally increase the plasma sodium concentration. This particular cycle may also be impracticable in those subjects, however, who have been overtreated with sodium and in whom any further sodium retention must be avoided. It seems apparent that no one universal program of cation exchange therapy can be presented for all patients with acute renal insufficiency. Usually, a combination of the acid and sodium cycles of the cationic resins provides the most satisfactory approach. The relative proportions of each cycle must be conditioned by the degree of acidosis and hyponatremia and by the amount of edema. Certainly, the hyperkalemia contraindicates the use of even small amounts of the K^+-resin.[16]

The experience with the use of the acid and sodium cycle resins in acute renal insufficiency is still somewhat limited. Nevertheless, in several patients these exchangers have achieved considerable reductions in the plasma potassium concentration.[16, 17] Their use is often complicated by the anorexia, nausea, and vomiting which are so frequent in acute renal insufficiency. Rectal irrigations of 10 per cent solutions have been suggested but by this route of administration their ultimate influence is far less predictable. Consequently, 40 to 60 grams per day must often be introduced by stomach tube. In the absence of adequate facilities for artificial dialysis, these resins, together with other conservative measures, may provide the sole effective approach for the control of progressive hyperkalemia.

Cortisone and ACTH Therapy

Therapy with cortisone and ACTH is often complicated by a conspicuous retention of sodium and water and moderate or severe hypokalemic, hypochloremic alkalosis.[18] The latter finding is usually prominent in those patients who are maintained on a liberal salt intake and who therefore require frequent mercurial injections to combat edema.[18] Such artificial diureses accelerate the loss of body potassium and chloride and thereby exaggerate any pre-existing hypochloremic alkalosis. As evident from FIGURE 1, the NH_4^+- or H^+-cycle should, in those patients eating a moderate amount of salt, reverse the disturbed pattern of hypochloremic alkalosis by increasing the plasma chloride and reciprocally reducing the bicarbonate concentration. In so far as the ingestion of this cycle will augment any potassium depletion, however, the degree of hypokalemia may be exaggerated. It has been demonstrated that potassium depletion prevents the correction of an underlying alkalosis.[19] In part, this circumstance may be explained by the inability of patients with severe potassium depletion to produce an alkaline urine, and by the effect of potassium depletion in producing a transfer of H^+-ion into the depleted cells.[20, 21] Consequently, the potassium depletion in patients on cortisone or ACTH must be counteracted by combining the acid resin with a considerable proportion of K^+-resin or with appreciable amounts of potassium by mouth. Despite the suggestion that adrenal activity may inhibit the effectiveness of these cation resins in increasing fecal sodium loss, studies on patients who are being treated with cortisone and ACTH have not revealed a marked resistance to the influence of these resins.[22] The combination of acid and K^+-resin may therefore prove of value in inhibiting any progressive salt retention in these subjects and in counteracting any significant degree of hypochloremic alkalosis.

Conclusion

It is apparent that the cationic exchange resins will prevent the absorption of appreciable quantities of ingested salt and simultaneously afford whatever benefits may be derived from a hyperchloremic acidosis. Under special circumstances, these resins may also provoke a dependable potassium loss. On the other hand, their use is complicated by the divergence of electrolyte changes elicited by varying the specific cation cycle. Alterations in dietary intake, reductions in renal function, and endogenous variations in adrenal function may further influence their ultimate effects. It has been shown that the sodium-withdrawing effect of prolonged usage may be less marked than that of short-term administration. The specific limitations and even the deleterious effects of the resins must constantly be borne in mind. No one pat formula can govern their use in each category of electrolyte disturbance. Every problem calls for an individual solution. Hospital pharmacies should maintain stores of each of the cation exchangers so that a judicious combination of resins may be administered whenever any of the commercial products are not suitable. These resins, augmented by whatever more effective exchangers become available, add an important new tool for the correction of fluid and electrolyte disturbances.

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Discussion of the Paper

Eugene Y. Berger, *Goldwater Memorial Hospital, New York University Medical School, New York*: There are two thoughts which come to mind in relation to the investigations which concerned the interchange of material between solution and solid exchanger. These thoughts were suggested by Doctor Waldo Cohn's and Doctor Isliker's work.

Dr. Waldo Cohn has demonstrated that nucleotides[1] will adhere to ion exchange resin. At an alkaline pH, nucleotides act as acids and adhere to anion exchange resins, and in a solution of acid pH, act as bases and adhere to cation exchangers. It has been known that when viruses or bacteria are placed in an electrical field the living matter will travel to the anode.[1] Dr. Gerald A. LoGrippo,[2, 3] using this information, has demonstrated that the Lansing virus in a 1 per cent suspension of central nervous system tissue at pH 6.6 will adhere to anion exchange resin (Amberlite XE-67 Rohm and Haas) and the virus may later be eluted, using the 10 per cent disodium acid phosphate, and still found to be infective. The Theiler virus and human poliomyelitis virus will also adhere to strong anion exchange resin and may be later eluted and found infective. It may be perfectly possible to isolate bacteria from solution using the same technique. The tubercle bacillus is attracted to the cathode[4] thus a cation exchange resin might separate it from solution.

Dr. Isliker has demonstrated that mixing out-dated red cells with cation exchange resin forms an insoluble antigen on to which antibodies will adhere. What the nature of the forces are which affix the blood cell stroma to the resin is not clear. It is doubtful that it is a simple form of ion exchange.

This procedure of mixing a solid ion exchanger with old blood has an interesting history and was described almost 2000 years ago, although not for the same purpose. I would like to quote from the Greek Herbal of Dioscorides as translated by Goodyear.[5]

"But that which is ye Lemnian earth is brought from the Island Lemnos, out of a certain hollow cavern having a marshy place. Thence it is gathered and is mixed with goats' blood, and the men there make it into trochisks, and sealing it with ye figure of a goat, call it ye seal of a goat. But it hath an eminent faculty of an antidote against deadly poisons when drank with wine . . . and it is good also for dysenteries."

C. J. S. Thompson in 1914 published a most wonderful history of these earths called *Terra Sigillata* or Sealed Earth.[6] *Terra Sigillata* in 1581 was subjected to scientific testing in that a condemned criminal received "a dram and a half of *Mercurie Sublimate*, mingled with conserve of Roses, and immediately after it he drank a dram of the *Terra Sigillata* in olde wine. And albeit the poison did . . . extremely torment and vexe him: yet in the end the medicine prevailing overcame it, whereby the poore wretch was delivered and being restored to his health was committed to his parents." Needless to say *Terra Sigillata* was quite popular in Italy in the 14th and 15th centuries, and I wouldn't be surprised if it were the antidote Lucrezia Borgia gave her son Gennaro. As late as 1900 bowls were made on the isle of Lemnos and were bought by the Turks who believed that a vessel made of this clay neutralizes the effect of any poison put in it. The last, Thompson reports of the hillside on the Isle of Lemnos, was that the proprietor wanted to plow over the spot and sow it with corn. It is delightful to read this article having the benefit of 20th century knowledge of alkaloids, electrolytes, medicine and ion exchange phenomena.

The suggestion that *Terra Sigillata* was (and is) an ion exchange material fits the story, but I think Thompson himself provides what I would consider quite adequate proof in the last paragraph of his article. "Some years ago I was fortunate to obtain a small sample of the Lemnian Earth of the sixteenth century." On analysis its composition was determined and found to be as follows:

Silicates	37.25 per cent
Ferric oxide	4.08 per cent
Aluminum oxide	13.51 per cent
Calcium oxide	22.90 per cent
Magnesia and alkali oxides	1.50 per cent
Water and carbon dioxide	17.72 per cent
Moisture as determined by heating at 130° in 100 parts	3.06 per cent

Although Thompson discounts this analysis as not meaning anything in particular, a present day chemist would be willing to accept the analysis as that of a zeolite.[7]

When an ion exchange resin, a solid, is placed in a beaker and solution containing electrolyte is added, there is an exchange of ions from the solution to the solid resin and equal exchange of ions from the resin to the solution. The exchange of ions between solution and solid is relatively easy to study in that the composition of the solution which is added to the resin in the beaker is analyzed before and after equilibration. There is no reason to believe that the principles which define the exchange of ions from solid resin to solution when the action is studied in a beaker do not also hold true when resin is taken into the intestinal canal. To hold the analogy, the dissimilarity is that the wall of the intestinal "beaker" is constantly losing ions from and adding ions to the system. The ions which remain on the resin when it is excreted are determined by the solution with which the resin last equilibrates. The nature of this solution is, in turn, determined by what the intestinal wall has added to or substracted from the system. Doctor Maurice Visscher in the 1940's carried out beautiful work with the use of radio-isotope tracers[8] to describe the nature of the wall of the "beaker." He has pointed out that the transfer of sodium, chloride, and water from the blood stream to the lumen of the intestinal tract and from the lumen back to the blood stream occur simultaneously and continuously. In the case of sodium, the rate of transfer of sodium from the lumen to the blood stream seems to be about the same throughout the length of the intestinal tract. The transfer from the blood stream into the lumen, however, occurs much more slowly as one proceeds from the duodenum to the colon so that in the colon the rate of transfer from the blood stream into the lumen is much slower than the transfer from the lumen into the blood stream. This results in a net reabsorption of sodium in the colon.[8]

If one would consider the colon analogous to the beaker, care must be taken in interpreting data because the nature of the beaker wall may be quite different from one human to another. It is still more difficult to interpret data from one species as applicable to another. Doctor McChesney has presented data which indicate that under normal circumstances the rat excretes the larger portion of the dietary sodium in the stool on low salt diets and it is not until the rat begins to eat a relatively high salt diet that appreciable portions of the dietary sodium begin to appear in the urine. This is quite different from man where, under normal situations, the amounts of sodium which appear in the stool are extremely small at all times. Thus, it would not be wise to use observations made in the normal rat to predict what may occur in feeding resin to normal man. In fact most of the data published indicate a rather large variability in the amount of sodium removed in the stool when cation resin is fed to different patients on the same dietary intake of sodium. When an edematous patient is fed resin, the amount of sodium removed in the stool is much less than that which is removed when the resin is fed to a patient without edema.[9] In other words the net transfer of sodium out of the colon wall back into the blood is much faster in the edematous patient than in the normal. The wall of the beaker then acts quantitatively different from human to human and from species to species.

The effectiveness of cation exchange resin in removing sodium from the body may be studied by measurement of the dietary intake and the fecal excretion of the sodium in a given subject. The nature and number of ions with which the resin comes into equilibrium as it passes from the oral to the anal end of the gastrointestinal tract may only be presumed. Thus far, two factors have been found, which will influence the net transfer of sodium out through the colon wall. These are desoxycorticosterone and the adrenal gland. Desoxycorticosterone will increase,[10] and adrenalectomy, as demonstrated by Doctor Kendall Emerson, will decrease the net transfer of sodium out of the colon.

The burden of development of resin for the treatment of edema has been placed on the chemist. With more recent insight into the physiological factors involved in the effectiveness of the resin, the burden is shifting to the physician. He has to find the means to alter the behavior of the colon wall, so as to decrease the net transfer of sodium out from the lumen to the blood stream. When, and if, he does manage to do so, he will probably be in a position to discard the use of resin for the treatment of edema after all.

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ION EXCHANGE RESINS AS A DIAGNOSTIC TOOL*

By Harry L. Segal

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Introduction

Indicator cation exchange compounds were introduced in 1950 by Segal, Miller and Morton[1] to determine gastric acidity without subjecting the individual to intubation. Further studies[2, 3, 4] have proved the efficacy of a quininium cation exchange compound as a tubeless gastric analysis technique to detect achlorhydria.

This paper will discuss the principles involved in the preparation, clinical application, and results of indicator ion exchange compounds in detecting gastric acidity without intubation.

Gastric analysis in clinical parlance refers to the study of gastric contents obtained through a tube introduced into the stomach. The degree of the acidity of the gastric juice so obtained is correlated with subjective and objective findings to aid in the clinical diagnosis. The acidity of the HCl secreted by the gastric parietal cells is constant and has been reported to be from 159 to 165 mEq./1.[5, 6] Yet, the acid concentration of the average gastric contents is usually less than 60 mEq./1 (pH 1.3). This is due mainly to the changing proportions between the parietal and non-parietal secretion in addition to any diluting effects of regurgitated duodenal juice, swallowed saliva, *etc.* Since the non-parietal secretion is more or less constant, the volume and acidity of mixed gastric juice is ordinarily the index of the secretory activity of the parietal cells.[7]

Custom has divided gastric acidity into four main clinical ranges (FIGURE 1), depending upon the mEq./1 (clinical units) of HCl output: hyperacidity [50–100 mEq. HCl/1 (pH 1.3–1.0)]; normal acidity [15–50 mEq. HCl/1 (pH 1.8–1.3)]; low acidity [$<$1–15 mEq. HCl/1 (pH 3.5–1.8)]; achlorhydria [1 mEq. HCl/1 (pH 3.5–7.0)]. Anacidity is a subdivision of achlorhydria and signifies no free HCl secretion with the pH varying between 7 and 8.5 depending upon the amount of gastric mucin in the gastric juice. The mEq. of free and combined HCl of the aspirated specimens are ascertained usually by neutralization with 0.1 *N* NaOH using Töpfer's reagent (dimethylaminoazobenzene) with its characteristic color change in the pH range 2.8–3.5 for free HCl and phenolphthalein with its color change in the pH range 8–10 as an indicator for total acidity. The mEq. or mg. of the HCl output per unit of time can be determined by measuring the volume of gastric contents obtained.

Indicator ion exchange compounds are comprised of ion exchange resins with special indicator ions which, when released from the compounds by an ion exchange reaction, can be readily identified in one or more of the body fluids. Two groups of such compounds have been prepared, one by replacing a specific number of mEq. of the H cations of a carboxylic or sulphonic cation exchange

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* Supported by a special medical research fund.

resin by an equivalent number of an indicator cation, and the other by adding a specific number of mEq. of an indicator anion to an anion exchange resin. The cation or an ion designated the indicator ion must be non-toxic, readily absorbed from the gastrointestinal tract after release from the compound, and easily detectable in the fluids in which it is tested. The relationship between the indicator ion eluted in the gastrointestinal tract by the H cations of the gastric juice of a specific pH and by the various cations in the small intestinal juices per gram of compound per 100 ml. of solution per unit of time, and that which will appear in the body fluid chosen for its detection during a specific interval after the oral administration of the compound, must be evaluated under normal and abnormal physiological conditions. With such information available, the manner of preparation and the oral dose of the specific indicator compound can be estimated to detect a specific degree of gastric acidity under proper conditions. Although both the amount of gastric secretion for a specific pH and the quantity of small intestinal secretion per unit of time can be approximately estimated, unpredictable variations in gastric emptying time and small intestinal motility make it impossible to estimate the exact number of mEq. of HCl per unit of time for any specific indicator exchange compound.

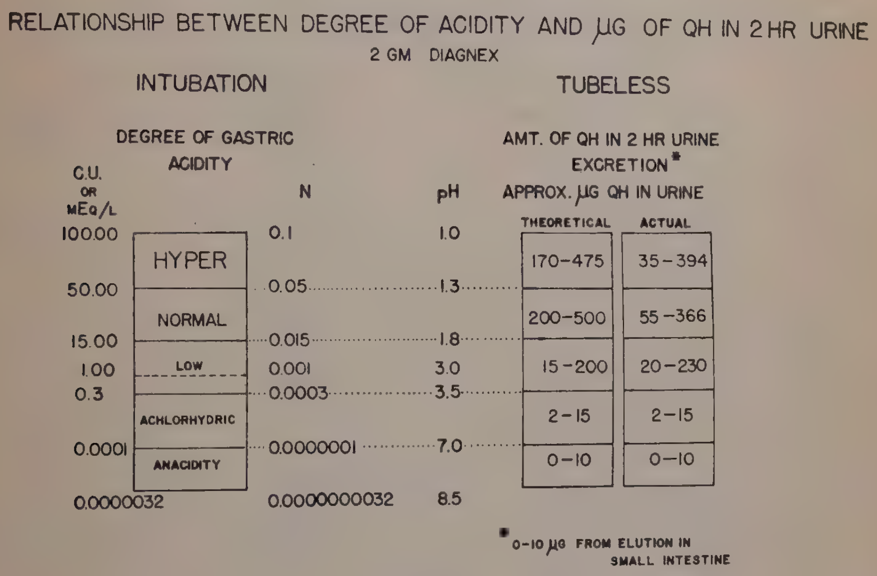

Figure 1.

A. Indicator Cation Exchange Compounds

1. Quininium Indicator Exchange Compound (*Diagnex*).* This compound is comprised of approximately 20–28 mg. (0.062–0.084 mEq.) of quinine as the indicator cation per gram of Amberlite XE-96* as the carboxylic cation exchange resin and has the general formula of

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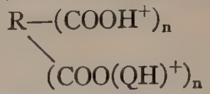
* Diagnex is prepared by E. R. Squibb & Sons, New York, N. Y.



$$R\text{—}(COOH^{+})_{n} \;\searrow\; (COO(QH)^{+})_{n}$$

Preparation. The cation exchange resin in the hydrogen cycle is washed down in a column with 2 *N* HCl followed by water until the eluate is neutral.

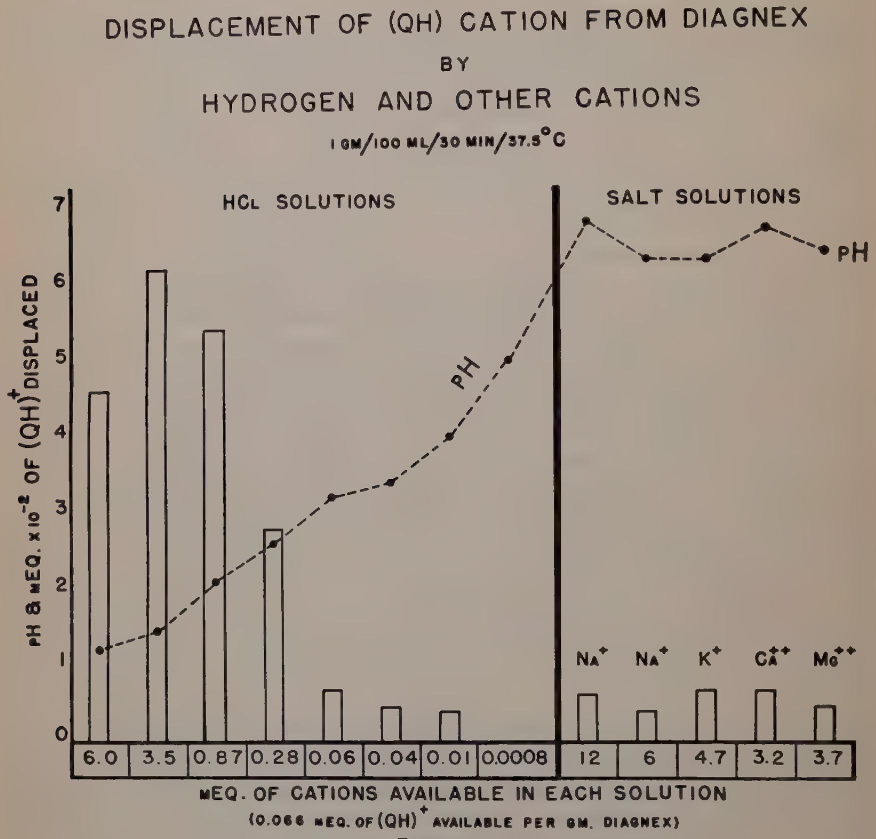


FIGURE 2.

A calculated slight excess of the amount of quinine as quinine hydrochloride per gram of resin is dissolved in water and this solution is passed through the column at a very slow rate. This is followed by water and washed until no more of the quinine hydrochloride is eluted. The original eluate in washings are pooled and analyzed. The amount of indicator cation retained by the ion exchange resin can then be calculated.

In vitro studies. FIGURE 2 reveals the amount of quinine eluted per gram of compound per 100 ml. of solution per 30 minutes at 37.5°C by the available

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* Amberlite XE-96 was generously supplied by Rohm & Haas Co., Philadelphia, Pa.

H cations of dilute HCl solutions of varying pH and of the various cations in salt solutions and especially of the cations in Ringer's solution which is more or less comparable to the cations available in the small intestinal secretions.

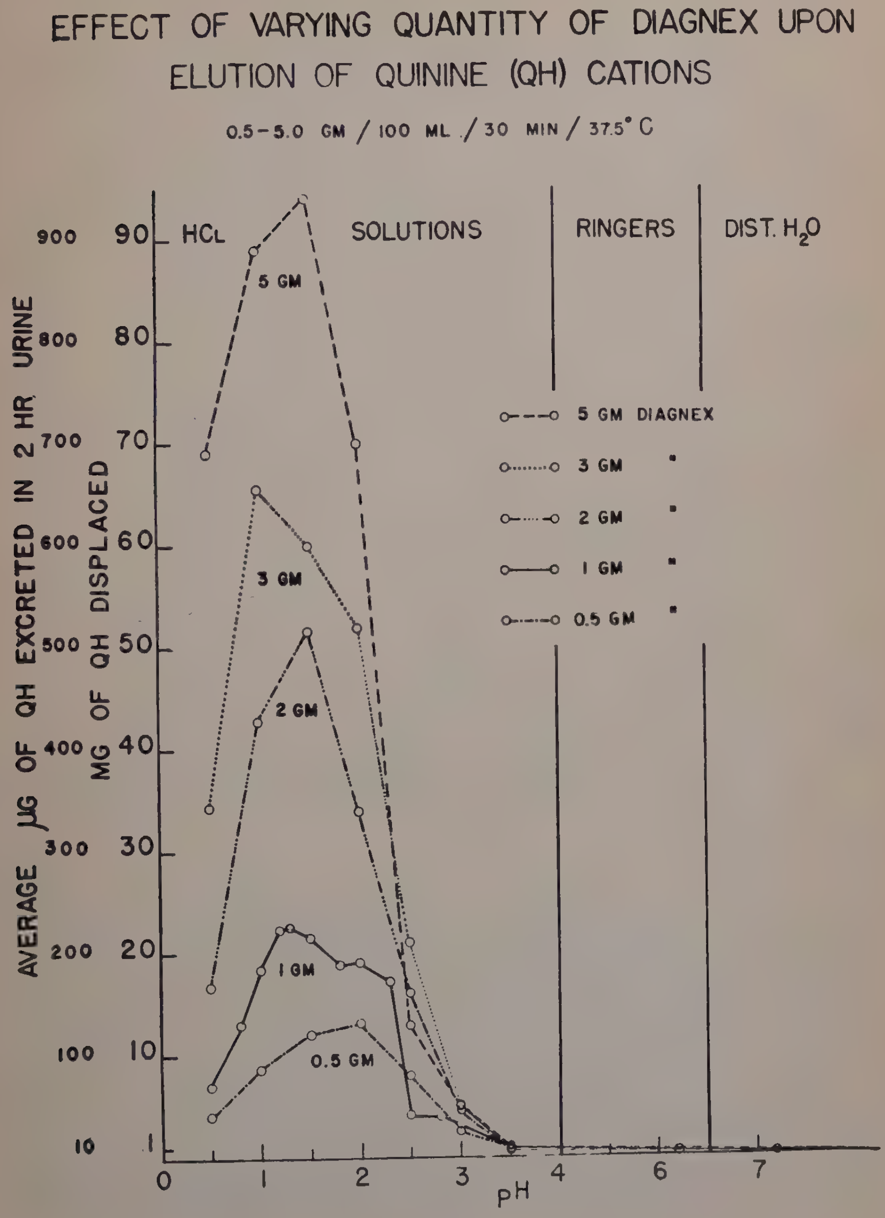

FIGURE 3.

For such elution studies 0.1 gram of the indicator exchange compound was stirred in 10 ml. of solution by a slow stream of air. The amount of quinine displaced was determined quantitatively by the use of a photofluorometer (Lumetron, model number 402EF, with primary filter B1).

Figures 3 and 4 reveal the relationship between the varying amounts of this indicator compound and constant volumes of the cation solutions and *vice versa*, respectively. These figures show that the per cent of elution of the quinine by cations in solution depends not only upon the pH but also upon the proportion of the indicator compound to the available cations in solution in the effective pH range. Referring to these figures, it is seen that 3 mg. of quinine will be released from 1 gram of Diagnex by the H cations present in 100 ml. of HCl solutions of a pH of 3 in 30 minutes at 37.5°C and only about 0.015 mg. of quinine will be eluted by the cations in 100 ml. or more of Ringer's solution under comparable conditions.

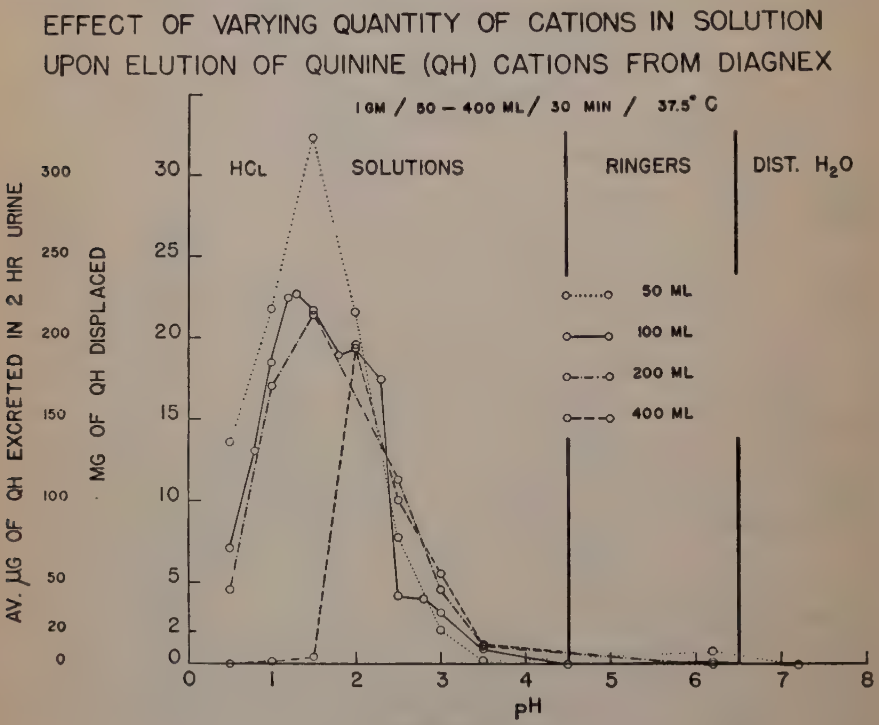
EFFECT OF VARYING QUANTITY OF CATIONS IN SOLUTION UPON ELUTION OF QUININE (QH) CATIONS FROM DIAGNEX

Figure 4.

Since studies have shown that an average of about 1 per cent of the quinine cations available in the gastrointestinal tract will appear in the first two hour urine excretion, approximately 0.030 mg. of quinine will occur in the first two hour interval as the result of the quinine cations displaced by the H ions of 100 ml. of gastric juice of a pH of 3 and only 0.0015 mg. of quinine in this same urine will be due to small intestinal juice activity under the same conditions. These facts allow the prediction of the oral dose of Diagnex necessary to detect gastric acidity of at least a pH of 3. Gastric acidity of a lower pH will result in an increased quantity of eluted quinine with a proportional increase in the quinine cations appearing in the urine regardless of the possible range of the gastric volume, while the amount of quinine secondary to any extremes of small intestinal volume will be of little moment in changing the per cent of quinine which will occur in the two hour urine excretion. The important consideration in estimating the oral dose of the compound is to allow for at least one gram of the compound to react with the gastric juice during a 30 minute period so that at least 3 mg. of quinine can be eluted at the lowest degree of acidity chosen for detection. Any calculated increase of quinine in the two hour urine resulting from the additive effect of constantly increasing volumes of small intestinal juice upon intact aliquots of the compound in its passage through the small intestinal tract will be roughly cancelled out by a corresponding decrease in the amount of quinine excreted in the urine as a result of the lessening time interval between the elution of the quinine in the small intestinal tract and its excretion in the two hour urine.

Both theoretical and clinical experience have shown that 2 grams of Diagnex is the ideal test dose for detection of free HCl at a minimum pH of 3.0–3.5. Another allowance in estimating the dose is the fact that, although the average per cent of quinine excreted in the two hour urine is 1 per cent, it can vary from 0.3 to 1.5 per cent in any single individual. If one-half of a 2 gram dose leaves the stomach intact to react in the small intestinal tract, sufficient compound would still remain in the stomach cavity to release enough quinine to give a positive urine test regardless of the fluctuations of the gastric juice volume. If the pH of the gastric juice should remain constant at 3 (which is unlikely) during the period allowed for ion exchange reaction in the stomach and if only 0.5 per cent of the eluted quinine appeared in the two hour urine excretion, the amount of quinine would be at least 15 gamma. Since the action upon the compound by the juices of the small intestinal tract will add another 2–10 μg of quinine in this urine, 17–25 μg of quinine would be present in the urine to be indicative of the secretion of gastric HCl of an acidity of at least a pH of 3. Since it is unusual for an acid secreting stomach to secrete gastric juice constantly at a pH of 3, a larger amount of quinine would usually occur in the two hour urine even in the low acid range.

FIGURE 1 demonstrates that Diagnex cannot be used in acid secretors to estimate the mEq. of HCl output per unit of time although the approximate amount of quinine excretion in the various acid ranges can be theoretically predicted under proper conditions. Since the emptying time of the stomach, the amount of quinine absorbed and varying abnormal states of the liver and kidneys can affect the amount of quinine which eventually will appear in the two hour urine excretion, the theoretical prediction will not be realized except in the low acid and achlorhydric ranges.

2. *Methylene Blue Indicator Exchange Compound.* A compound similar to Diagnex has been made comprising 34.5 mg. of methylene blue per gram of

Amberlite XE-96. Elution studies of this compound (FIGURE 5) demonstrate characteristics similar to those of the Diagnex compound except that the number of methylene blue cations displaced is less than the quinine cations released from Diagnex under comparable conditions. FIGURE 5 shows that 1.5 mg. of methylene blue will be eluted from 1 gram of this methylene blue indicator compound by the H cations of 100 ml. of HCl solution of a pH of 3 during 30 minutes at 37.5°C and that 0.95 mg. of methylene blue will be displaced by the cations in 100 ml. of Ringer's solution under comparable conditions.

Since preliminary studies have shown that about 5 per cent of the methylene blue cations available in the gastrointestinal tract will appear in the first two hour urine excretion, approximately 0.075 mg. of methylene blue will occur in the first two hour urine excretion as the result of the methylene blue cations displaced from 1 gram of the methylene blue indicator compound by the H cations of 100 ml. of gastric juice of a pH of 3 and less than 0.05 mg. of methylene blue in the urine will be due to small intestinal juice activity under the same conditions. These facts allow the prediction of the dose of this compound necessary to detect gastric acidity of at least a pH of 3–3.5.

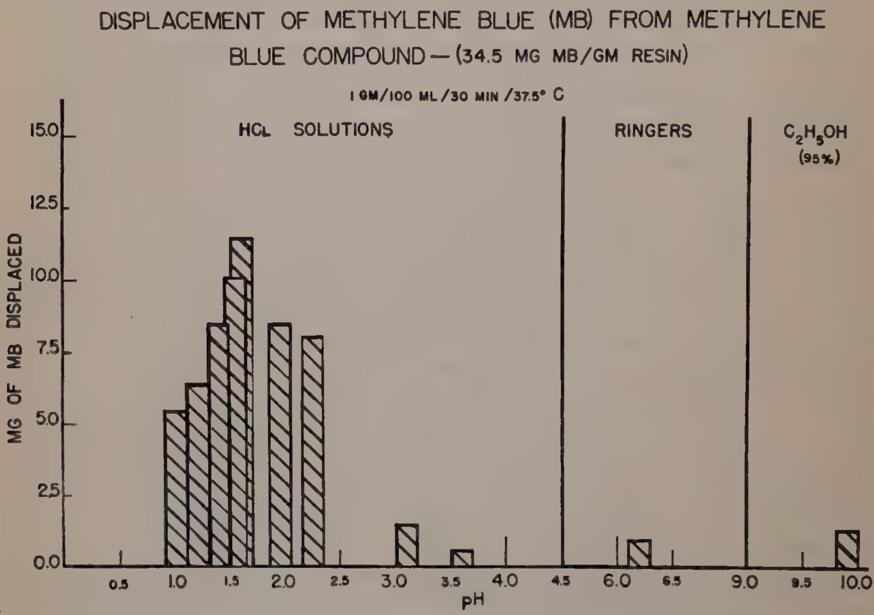

FIGURE 5.

3. Indicator Exchange Compounds with Azo Dye Cations. Two such compounds have been prepared with serenium and pyridium as the respective indicator cations and Amberlite XE-96 as the cation exchange resin. FIGURE 6 suggests that the serenium indicator compound has characteristics similar to those of Diagnex with the important exception that 95 per cent ethyl alcohol can release all the available serenium from this compound. This indicates that the serenium in this compound is attached to the resin by adsorption forces.

The compound made with 36 mg. of pyridium per gram of Amberlite XE-96 reacts similarly to that of the serenium indicator exchange compound described.

4. *Serenium-Permutit-H* Indicator Exchange Compound.* This indicator cation exchange compound, comprised of 40 mg. of serenium per gram of Permutit-H, a cation exchange resin, has been prepared in a manner similar to that already described for the preparation of the other indicator cation exchange compound. FIGURE 7 shows that about 0.7 mg. of serenium is released from 1 gram of this Serenium-Permutit-H indicator compound by the action of the H ions in 100 ml. of HCl solution of a pH of about 1.1, and slightly less than 0.4 mg. of serenium is released by the H ions of 100 ml. of HCl solution at a pH of 1.8. Only about 0.1 mg. of serenium is eluted by 100 ml. of HCl solution of a pH of 3 or by 100 ml. of Ringer's solution.

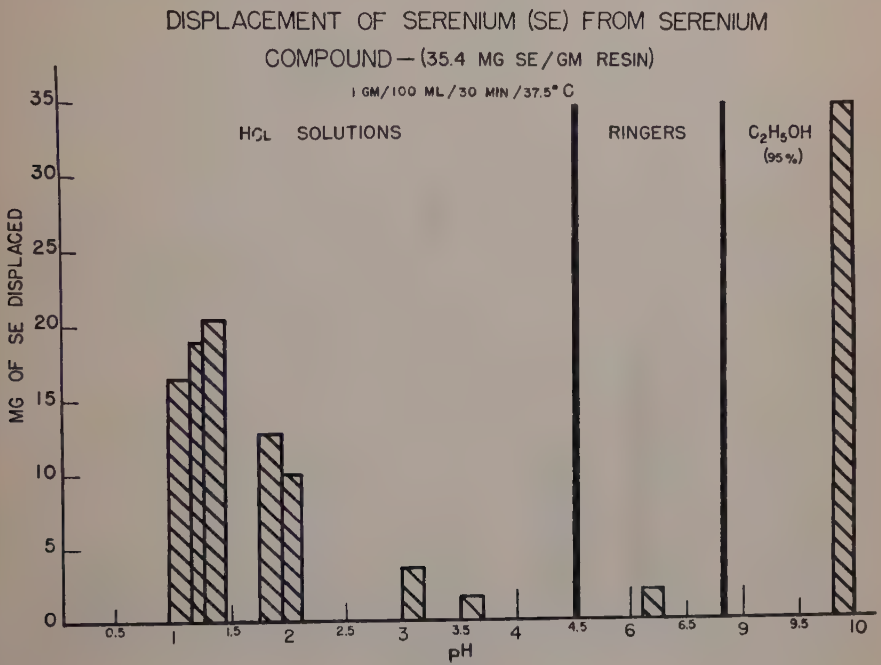

FIGURE 6.

Approximately 5 per cent of serenium cations available in the gastrointestinal tract appear in the first two hour urine excretion. It is evident from FIGURE 7 that, after the oral administration of 2 gm. of this compound, approximately 0.035 mg. of serenium should occur in the first two hour urine as the result of H cation activity of 100 ml. of gastric juice of a pH of 1.1, as against 0.02 mg. of serenium from the activity of 100 ml. of gastric juice of a pH of 1.8 and 0.005 mg. of serenium in the two hour urine elution serenium from both 100 ml. of gastric juice of a pH of 3 and 100 ml. of small intestinal juice. Although such amounts of serenium in the urine are insufficient for accurate clinical detection, the facts presented allow the prediction of the preparation of indicator compounds to detect different degrees of gastric acidity.

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* Permutit-H was generously supplied by The Permutit Co., New York, N. Y.

B. Indicator Anion Exchange Compounds

1. Fluorescein Indicator Anion Exchange Compound. The compound has been made by the addition of fluorescein as the indicator anion to Amberlite IR-4B as the anion exchange resin so that 1 gram of compound contains 33 mg. of fluorescein. This compound has been prepared as follows. The IR-4B analytical grade was converted to the hydrochloride form by the slow addition of 4 per cent HCl to the resin in a batch process. The mixture was stirred for 15 minutes. The acid layer was decanted and a similar amount of HCl was again mixed with the resin. The assayed layer was decanted and an equal amount of distilled water was then added to the resin, the mixture being agitated for 10 minutes. The water layer was decanted and the process repeated until the decantate showed a pH of two or more. A solution of U.S.P. fluorescein was added slowly to the Amberlite IR-4B* resin to which distilled water had been added. The mixture was agitated five hours, filtered and washed

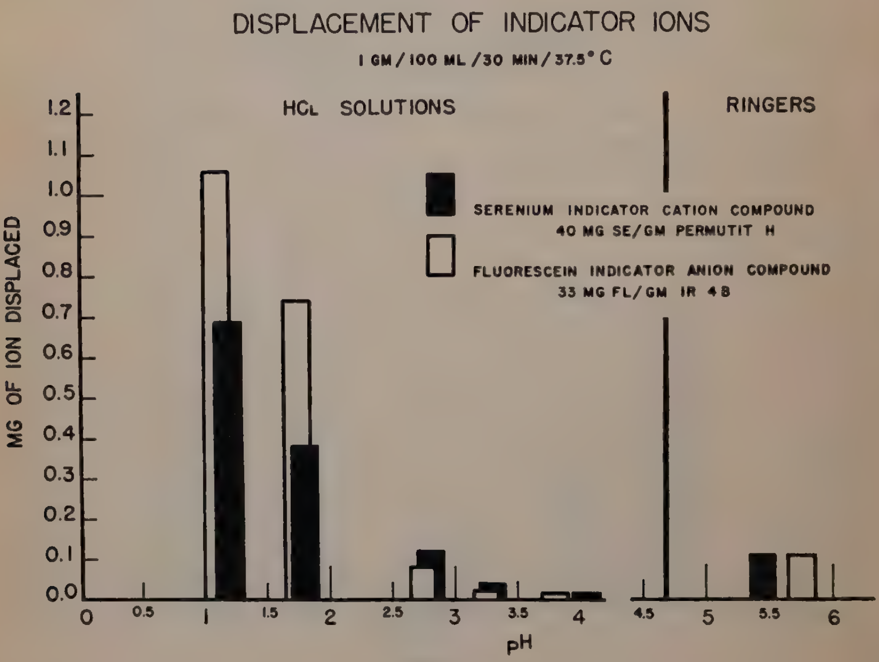

Figure 7.

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[*] Supplied by Rohm & Haas Co, Philadelphia, Pa.

until the washings no longer showed any trace of fluorescein. The compound was dried with absorbent paper and then over calcium chloride.

FIGURE 7 demonstrates that the characteristics of this compound are similar to those of the Serenium-Permutit-H indicator compound. The percentage of the fluorescein available in the gastrointestinal tract that will appear in the blood or urine during a specific period of time has not yet been investigated in this laboratory. This compound also suggests the possibility of the preparation of an indicator anion exchange compound capable of detecting a specific range of gastric acidity.

Clinical Studies

The quininium indicator exchange compound (Diagnex) has been tested in more than 1000 individuals*, 400 of whom have had their gastric acidity determined by the intubation technique.

There were four false negative tests in the 270 individuals who secreted free gastric HCl and five false positive results in 100 achlorhydric control patients. The tubeless gastric analysis findings were variable in a group of 30 who were achlorhydric to alcohol or caffeine but secreted free HCl after histamine stimulation. In two of the five individuals with false negative findings who were checked, the repeat tubeless gastric analysis showed the presence of free HCl. The five individuals with false positive tests excreted between 15 and 30 gamma of quinine in the two hour urine and should really be labeled as low acid secretors instead of false positive. Three of these individuals in whom the tubeless gastric analysis was repeated showed less than 15 gamma of quinine in the two hour urine excretion. Such variations may be due to technical errors but more likely to the fact that the gastric secretion of HCl is especially variable in individuals in the low acid range. A majority of individuals in the special group of 30 who were achlorhydric after alcohol or caffeine but not after histamine stimulation revealed free gastric HCl by the tubeless technique. This is probably due to the fact that these patients secreted sufficient HCl during the period of the two hour urine collection to release enough quinine in the gastrointestinal

* The clinical test is performed by the administration of 2 grams of Diagnex after proper stimulation with a gastric stimulant and collecting the urine two hours after the oral administration of the compound. The urine is assayed for quinine by a simplified modification[4] of the Kelsey-Geiling ether-sulphur acid technique,[8] the prin- ciples of which are as follows. Ether is added to the urine made alkaline with normal NaOH. Ninety-five per cent alcohol is occasionally necessary to break an emulsion. The quinine is removed from the ether by 0.1N sulphuric acid. The amount of quinine can be determined accurately with a photofluorometer or semi-quantitatively by comparing it with standards subjected to ultra-violet rays.

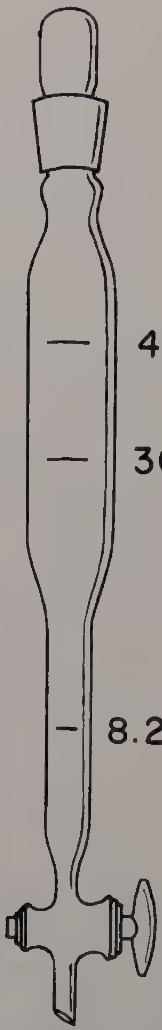
The exact procedure is as follows. The urine is diluted with distilled water to a volume of 300 cc. Thirty cc. of this mixture (10 per cent of the volume) is transferred into a special separatory funnel (see FIGURE 8). From 0.5 to 1 cc. of normal NaOH is added to elevate the pH to 11.5 or more. Fifteen cc. of ether is then added, bring- ing the mixture to the 45.5 cc. mark on the funnel. After shaking the mixture gently, a few drops of 95 per cent alcohol are added if an emulsion is present. The urine and ether layers are drawn off to the 8.2 cc. mark which allows 8.2 cc. of ether to remain in the separatory funnel and represents about 5 per cent of the original quinine in the urine. Five cc. of 0.1N sulphuric acid is mixed with the ether remaining in the funnel and then the lower sulphuric acid layer is drawn off into a test tube and examined for quinine as described.

When the urine volume exceeds 300 cc. the total volume is measured and a 30 cc. aliquot without dilution is tested. The quantity of quinine in the aliquot is multiplied by the fraction: $\frac{\text{total volume ml.}}{\text{300 ml.}}$ to obtain the total quinine in the voided urine.

The Blak-Ray Lamp, Model X-4, was used in this study. FIGURE 9 shows a proper housing for enclosing for daylight use.

It is necessary to keep all reagents in glass bottles with glass stoppers. Rubber or plastics can produce fluorescence.

Interpretation. Free acid is present if the two hour urine excretion contains more than 25 micrograms of quinine. Free gastric HCl is absent if the two hour urine excretion contains less than 15 micrograms of quinine. If the amount of quinine excreted in the two hour urine ranges between 15 and 25 micrograms the individual is considered to be a low acid secretor.



Type of Separatory Funnel for Quinine Assay.

Figure 8.

tract for the excretion of more than 15 mg. in the two hour urine. Those in this group who failed to show free gastric HCl by the tubeless method usually secreted less than 10 mEq. of free HCl/1 after histamine stimulation as tested with the intubation technique.

Although 250 mg. of caffeine sodium benzoate is an adequate stimulus in the average case, some individuals secrete free gastric HCl only after stronger stimulants. This has occurred in a few individuals whose tubeless gastric analysis results showed no free HCl with 250 mg. caffeine sodium benzoate but revealed free HCl with 500 mg. of caffeine sodium benzoate or histamine as the gastric stimulant. Thus, in certain conditions either 500 mg. of caffeine sodium benzoate or histamine may be desirable as the stimulant.

The pitfalls inherent in tubeless gastric analysis must be kept in mind. In the presence of pyloric obstruction the eluted quinine may not enter the small intestine in sufficient amount to give an accurate index. Similarly, disturbances of the liver and kidney may interfere with the normal rate of urinary quinine excretion. It is obvious that medications such as quinine, quinidine or atabrine will interfere with this test. Harkness[9] has suggested that drugs containing cations such as aluminum, magnesium, calcium and iron should be discontinued 24–48 hours before this test as any of these cations remaining in the stomach can release the quinine and be the basis for a false positive test

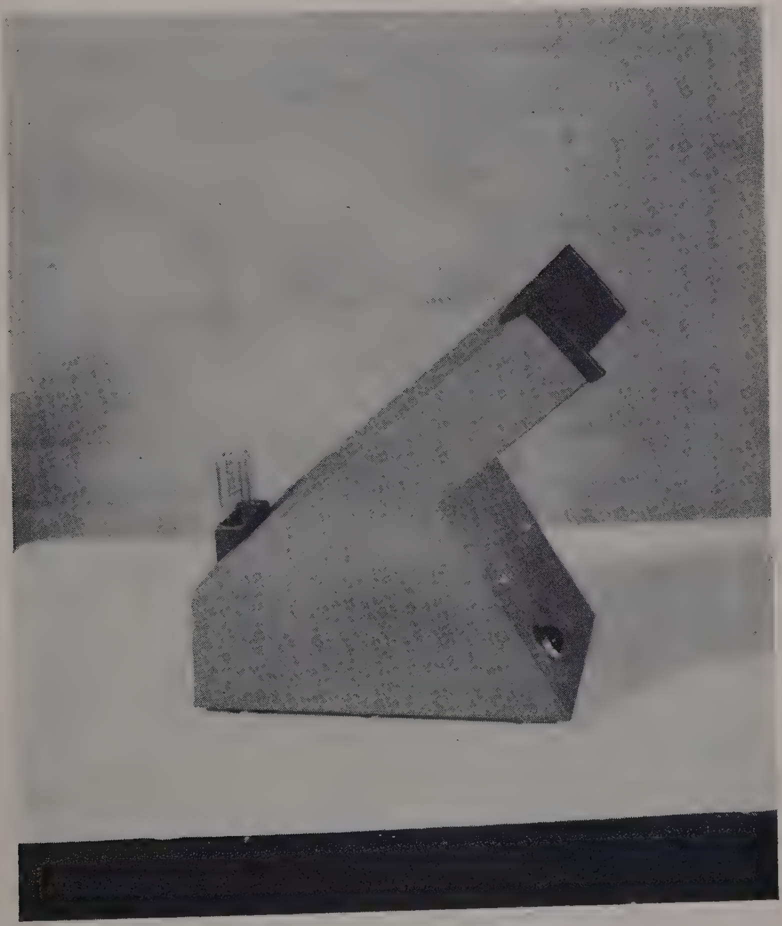


FIGURE 9. Housing for Blak-Ray Lamp for daylight use.

in the absence of free HCl. Since ether also extracts riboflavin and nicotinic acid derivatives, such vitamins should be avoided 24 hours before the quinine assay.

In order to simplify the test for more general use, compounds containing a cation which, on excretion in the urine, will detect the presence of free gastric HCl by a color change in the urine have been studied and are still under investigation. FIGURES 5 and 6 demonstrate the theoretical possibility of the use of such compounds to determine the presence or absence of free gastric HCl by a mere change in the color of the urine.

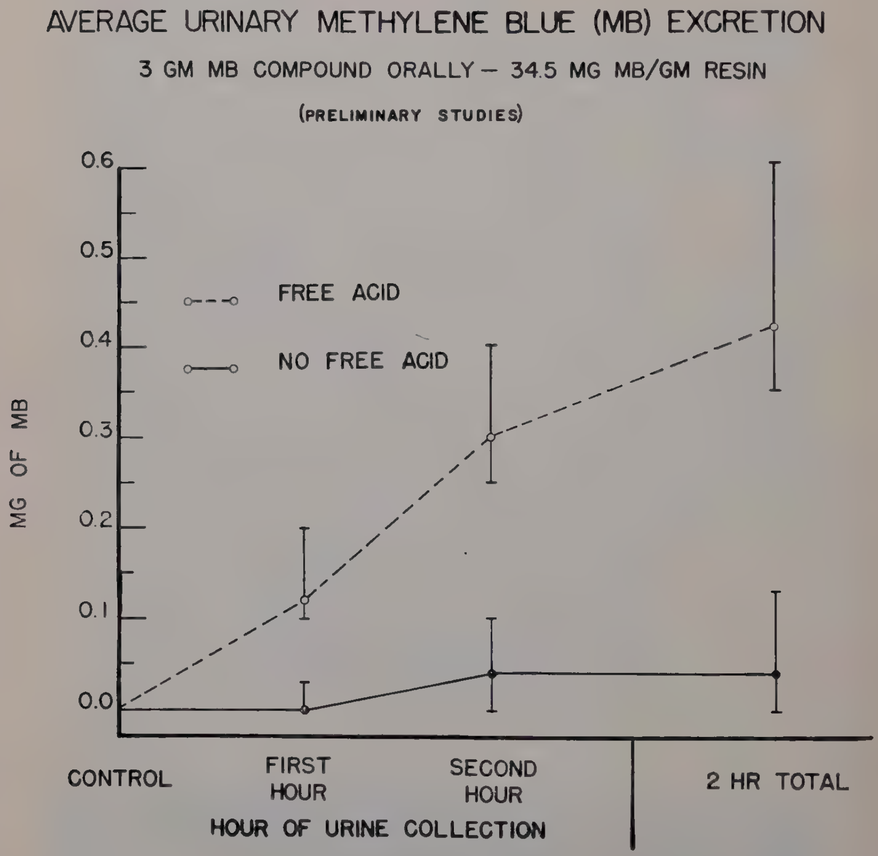


FIGURE 10.

Preliminary clinical studies (FIGURE 10) show that the oral administration of 3 gm. of methylene blue compound will average more than 0.4 mg. of methylene blue in the first two hour urine excretion in individuals who secrete free gastric HCl, as against an average of less than 0.05 mg. of methylene blue in the achlorhydric group. This verifies clinically the theoretical *in vitro* predictions (FIGURE 5) and suggests the eventual use of this and similar compounds as a simple diagnostic tool to detect gastric acidity by a color change in the urine. Simple color standards can be used to estimate the amount of these cations in the urine.

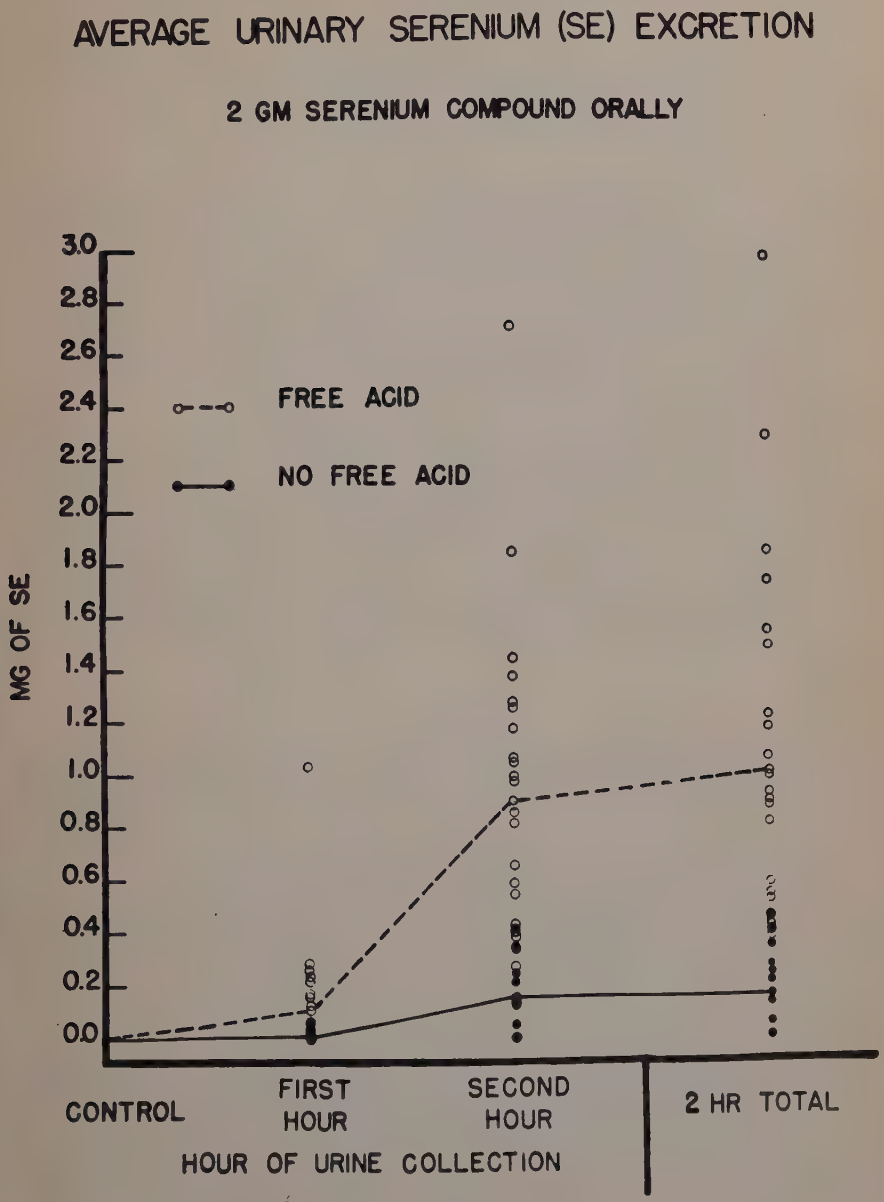


FIGURE 11.

A mixture of Diagnex and the methylene blue indicator compound are under clinical trial with the idea of omitting the quinine assay when the color in the urine due to methylene blue signifies the presence of free HCl. In the absence of a significant color change, the urine will be assayed for quinine.

Similarly, preliminary clinical investigation with 2 gram doses of the serenium indicator compound (FIGURE 11) has revealed that more than 0.4 mg. of serenium was excreted in the two hour urine in the presence of free gastric HCl, and less than 0.25 mg. of serenium in the absence of free HCl. Because of the possible release of the serenium of this compound by a reaction other than ion exchange and the uncertainty of the presence of free gastric acid in the urinary excretion range between .25 and 0.5 mg. of serenium, the modification of this compound is being investigated to meet these objections. Mixtures of this compound with Diagnex are being studied to learn whether the color change in the urine due to more than 0.4 mg. of serenium will be consistently indicative of free gastric HCl. This will similarly obviate quinine assay as already described for the mixture of Diagnex and the methylene blue compound.

The pyridium indicator compound, the Serenium-Permutit-H indicator compound, and the fluorescein indicator anion exchange compound have not yet been studied clinically.

The investigations reported show that it is now possible by the use of indicator ion exchange compounds to detect the presence or absence of free gastric HCl without subjecting the individual to intubation. The fact that the elution of the indicator ion of an ion exchange indicator compound depends upon certain inherent properties such as the pH of the solution, molecular weight, valence, and concentration of the ions in solution and the acid capacity of the ion exchange resin makes it possible to prepare and predict the efficacy of specific indicator compounds to estimate degree of gastric acidity without intubation.

Summary

The rationale of the preparation and use of indicator exchange compounds to determine gastric acidity without subjecting the individual to intubation have been described.

In vitro studies of four different indicator cation exchange compounds with clinical results in three of the compounds are reported.

The use of the indicator exchange compound called Diagnex in more than 1000 individuals has proved the efficacy of this technique. Clinical investigations of a methylene blue indicator compound and of a serenium indicator compound show promise of estimating the ability of the stomach to secrete free HCl by a mere color change in the urine.

In vitro studies of an indicator cation exchange compound comprised of serenium as the indicator cation and Permutit H as the cation exchange resin, and of an indicator anion exchange compound with fluorescein as the indicator anion and Amberlite IR-4B as the anion exchange resin, suggest the possibility of the preparation of various indicator exchange compounds to detect ranges of gastric acidity.

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